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ERRATA AND AUTHORS' EMENDATIONS

Page 43, par. 2, line 2, "figure 6" should be "figure 5."

Page 77, second equation should be $V_1 = V_0 e^{\frac{\mu}{2}\left(\frac{T_1 - T_0}{T_0 T_1}\right)}$.

Page 168, line 6, "the integral of $f_1(q)$" should be "the integral of $1/f_1(q)$."

Page 168, par. 2, line 8, "P^1_f" should be "P'_f."

Pages 275 and 287, the graphs shown as figures 1 and 7 should be interchanged; legends are correct as they stand.

Page 298, third line from bottom, "1:ratio" should be "1:2 ratio."

Page 301, title, "Acid" should be "Acids."

Pages 309 and 310, the photographs shown as figures 1 and 2 should be interchanged; the legends are correct as they stand.

Page 315, par. 4, line 3, "Illinois Large Podded" should follow "Baby Potato," and "Henderson Bush" should follow "Illinois Large Podded."

Page 319, par. 2, line 9, "solution" should be "dilution."

Page 323, table 7, first figure column, in line 12 (for Pencil Pod Black Wax) and in line 16 (for Striped Creaseback (Scotia)) "+" should be "±."

Page 326, par. 5, line 6, "8 heterozygous" should be omitted and "24 susceptible" substituted.

Page 333, table 1, last figure in last column, "233.35" should be "223.35."

Page 400, table 6, "*Percent*" should be deleted from 4th column, entitled "Difference."

Page 461, table 2, col. 4, last two figures, "40" should be "36" in each case.

Page 461, 5th line from bottom, "40" should be "36."

Page 497, table 7, col. 6, mean bursting strength for fabric No. 75, skirt No. 2, given as "66.25" should be "66.65."

Page 508, table 2, last two lines, "B^1" and "B^2" should be "B_1" and "B_2."

Page 509, equation at bottom of page, "24" and "48" should be large type like other digits in this equation.

Page 511, first equation, "$B^2/_2$" should be "$B_2/2$."

JOURNAL OF AGRICULTURAL RESEARCH

Vol. 67 Washington, D. C., July 1, 1943 No. 1

ANATOMICAL AND OTHER STUDIES ON MAZZARD CHERRY SEEDLINGS HAVING EXCESSIVE ROOTS AT THE COLLAR REGION [1]

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INTRODUCTION

Malformations characterized by an excessive number of roots at the collar region of cherry trees were observed in a nursery in 1929. The condition was noted on approximately 3 percent of the trees that had been budded on mazzard cherry (*Prunus avium* L.) seedlings. These tufts of roots (fig. 1, *A*) on the seedling part of the tree presented such an abnormal and unsightly appearance that the grower discarded the trees. Although these malformations bore a superficial resemblance to the hairy root disease caused by *Phytomonas rhizogenes* Riker et al., common on apple (*9*),[3] they were not typical of this disease, and routine pathological studies failed to disclose a causal organism.

Several years later, excessive roots just below the ground level were observed on a small percentage of 1-year-old mazzard seedlings grown at Beltsville, Md. These tufts of roots (fig. 1, *B* and *C*), in contrast with normal rooting (fig. 1, *D*), were obviously an early expression of the condition that had been observed previously on the nursery trees. As the presence of numerous root primordia in the young hypocotyl obviously would account for the excessive-root malformations observed on the trees in the nursery, anatomical studies were made on young mazzard cherry seedlings. Such studies are of considerable importance in supplying information for interpreting growth responses in plants (*3*).

Among horticultural workers, Priestly and Swingle (*8*), in particular, have emphasized the value of and the need for anatomical studies as a basis for explaining the vegetative responses of certain plants propagated by root or stem cuttings. Their detailed studies and their extensive review of the literature on the role of adventive root and shoot primordia in vegetative propagation provide an excellent background for workers interested in studying the ontogeny of these organs. Information of practical value is accumulating as a result of anatomical analyses that have served to explain such responses as the rooting potentialities of etiolated shoots (*7*), regeneration in stem and root cuttings (*2, 5, 6, 13*), and the dwarfing effect of certain rootstocks (*1*). On apple, anatomical studies have been made on the origin of root and shoot primordia in stem tissue (*5, 14*) and in roots, particularly on regeneration (*12*) and the initiation of primordia (*10*).

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[1] Received for publication May 22, 1942.

[2] The writer is indebted to J. J. Bowman, of this Division, for assistance in preparing the slides and photomicrographs for these studies.

[3] Italic numbers in parentheses refer to Literature Cited, p. 15.

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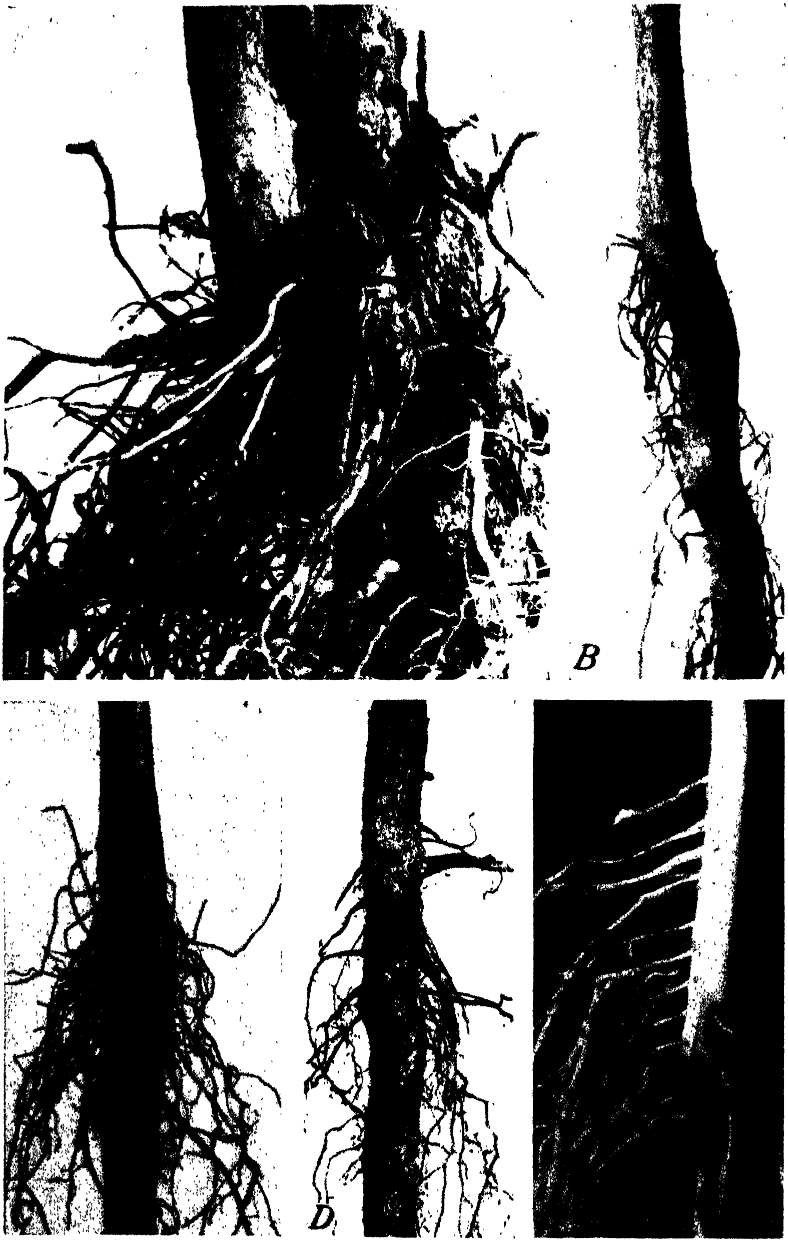

FIGURE 1.—Root development on the collar region of mazzard cherry: *A*, Tufts of roots on a 1-year-old budded tree; *B* and *C*, excessive roots on 1-year-old seedlings; *D*, root development on a normal 1-year-old seedling; *E*, linear arrangement of primary roots on a 6-week-old seedling (slightly enlarged).

The anatomical studies reported herein were intended mainly to supplement the present information on the origin and development of root primordia in fruit trees (*4*). An effort was made to identify early stages of root primordia and to note any synchronization of the initiation of primordia with the ontogenetical development of the parent tissue (*4, 8*). The limited pathological studies reported were made mainly to eliminate the suspicion that the hairy root organism may be a causal factor in the development of these excessive roots.

MATERIAL AND METHODS

In the pathological studies, routine methods in isolation and in inoculation tests were used. Supplemental observations were also made on seedlings grown in steamed soil.

For the anatomical studies, mazzard seeds were germinated and the seedlings were grown in the greenhouse in order to observe the occurrence of excessive roots. When the seedlings had made shoot growth ranging from 8 to 15 cm., portions of the hypocotyl and root were killed in formalin-acetic-acid-alcohol solution. The material was later embedded in paraffin, sectioned, and stained in the usual histological routine, except that butyl alcohol was frequently used in the killing as well as in the dehydrating series.

OBSERVATIONS AND RESULTS

PATHOLOGICAL EXPERIMENTS

The results of the limited pathological experiments and observations are summarized briefly. The malformations (fig. 1, *A*) not only are atypical of the hairy root disease, but also they occur in districts where the hairy root organism is not commonly found. The general aspect of these excessive root formations more closely resembles the genetic condition known as noninfectious hairy root on apple (*11*), but there is no evidence that the condition on cherry is genetic.

Attempts to isolate the hairy root organism from affected trees over a 5-year period were unsuccessful except in one instance, which was considered fortuitous because of previous experience with this organism as a surface contaminant (*9*). Inoculations with the hairy root organism into the collar regions of 2-month-old mazzard cherry seedlings growing in previously steamed soil, however, resulted in infection on 13 of the 15 seedlings that were inoculated; the 10 punctured, noninoculated seedlings remained normal. The symptoms on these infected seedlings were typical of the early stages of hairy root on apple and were characterized by dense aggregations of root "knobs"; the organism was reisolated. Although this evidence establishes mazzard cherry as a host for the hairy root organism, it also indicates that the type of malformation under consideration in this paper is not the result of infection by this organism. Because the possibility that the condition is the result of infection by a bacterium or a fungus appeared so remote, subsequent attention was confined to morphological and anatomical features as a supplement to previous studies on the origin of adventive primordia (*10*).

MORPHOLOGICAL AND ANATOMICAL FEATURES

VASCULAR ARRANGEMENT

The hypocotyl of mazzard cherry seedlings seldom exceeds 7 cm. in length; under conditions favorable for extension, the maximum observed length was 9.5 cm. Under greenhouse conditions favorable for vigorous growth, some of the young seedlings exhibited a longitudinal splitting in the cortex of the hypocotyl (fig. 1, *E*). Lateral roots also frequently made their appearance normally in vertical rows on the hypocotyl. This linear arrangement of course indicates a morphological relation of these roots to the xylem in the hypocotyl (*3*, *14*). As expected, adventive root primordia were found in such seedlings in sufficient numbers to account for the masses of roots that sometimes occur on older nursery trees (fig. 1, *A*) and on 1-year-old seedlings lined out for budding (fig. 1, *B* and *C*).

The illustrations in figure 2 are transverse sections of a seedling showing the vascular arrangement at different zones in the root and hypocotyl of a seedling beginning secondary growth. The relative size of the vascular cylinder conforms with the gross appearance of seedlings, which at this stage generally are considerably enlarged at the base of the hypocotyl.

In the root (fig. 2, *A*), the xylem cylinder is established and the epidermal cells are collapsing, preliminary to the disintegration of the cortex.

At the base of the hypocotyl (fig. 2, *B*), the xylem cylinder is interrupted by four gaps, resulting in four arcs of xylem. Successive sections up the hypocotyl show a progressive tendency toward forking into two separate strands at the center of each primary xylem group. At the zone shown, tangential divisions in the layer of cells of the pericycle inside the endodermis indicate the initiation of the phellogen. The zone 7 mm. farther up the hypocotyl (fig. 2, *C*) is characterized by a definite gap in the center of each arc of xylem. At this level gaps in the procambium strands from the stem apical meristem occur over the four primary xylem regions. Procambium activity between the four "paired" xylem strands results in xylem differentiation, which serves to unite one strand from each of the "paired" xylem strands with the adjacent strand of the next pair. This general type of orientation in the transition zone corresponds with "type A" of Eames and MacDaniels (*3*). At this stage the outline of the xylem is roughly in the form of a square. The four-sided appearance is accentuated by the presence of thick-walled parenchyma in tangential lines over the cambium. These thick-walled parenchyma cells possibly serve as protective tissue previous to the differentiation of four arcs of fibers, which at a slightly later stage (fig. 3) form a cap over the phloem elements.

FIGURE 2.—Transection showing vascular arrangement at different levels in a young seedling beginning secondary growth: *A*, Root tissue just below base of hypocotyl; *B*, tissue at the base of the hypocotyl, 1.1 mm. above *A*; *C*, tissue in a zone 7 mm. farther up the hypocotyl. All × 80. *e*, epidermis; *en*, endodermis; *p*, pericycle; *par*, thick-walled parenchyma; *r*, lateral root primordium; xy_1, region of primary xylem.

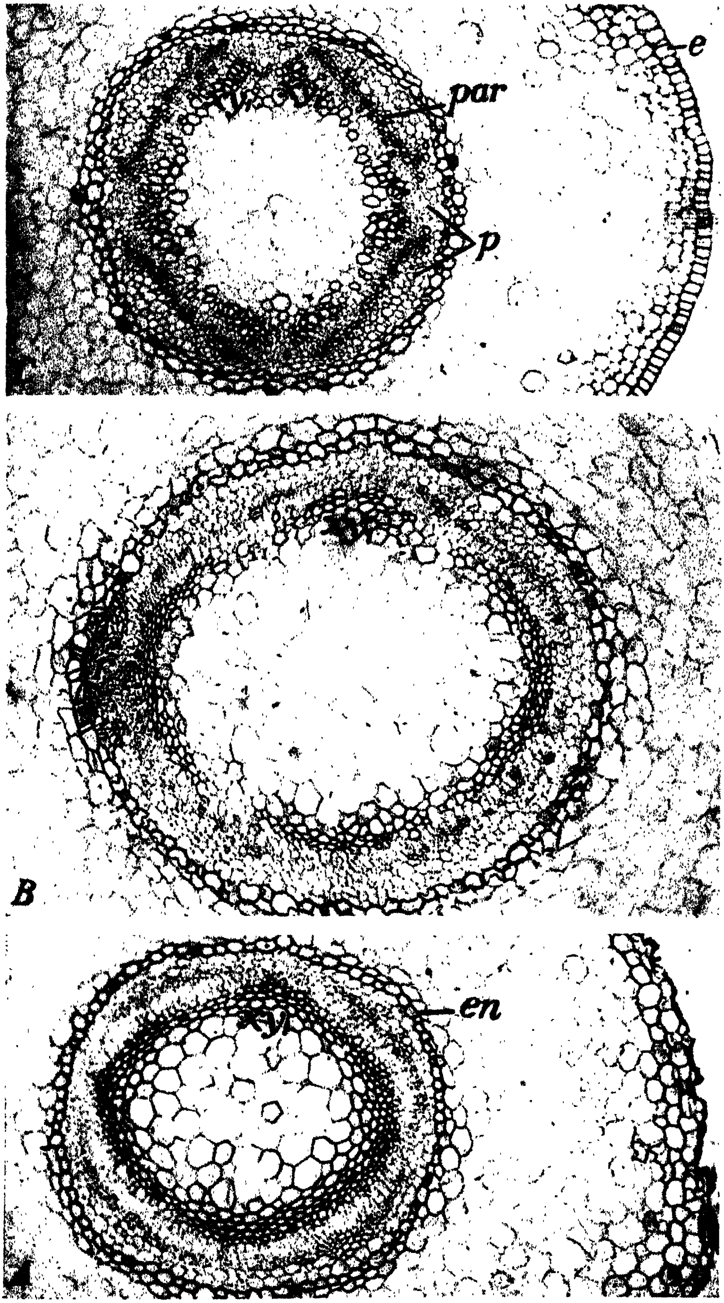

FIGURE 2.—For explanatory legend see opposite page.

LATERAL ROOTS

The origin and development of lateral roots are described because some such roots are undoubtedly intermingled with adventive roots in the tufts of roots under discussion.

Lateral roots develop in the pericycle over the general region of the primary xylem at the root-stem junction (fig. 2, *B*) and in the hypocotyl (fig. 3). As a result, these roots appear in vertical rows. The primordia shown in figure 4, *A* and *B*, are developing at the base of the hypocotyl. At this comparatively advanced stage many cells in this meristematic area have large deep-stained nuclei. Activity of this type occurs over a comparatively large area (fig. 4, *A*) even including the enveloping endodermis. The meristematic endodermis persists as an enveloping layer in at least considerably later stages than that shown in figure 4, *B*.

When conditions of growth are such that the individual strands in each of the "paired" xylem strands are widely separated, lateral roots

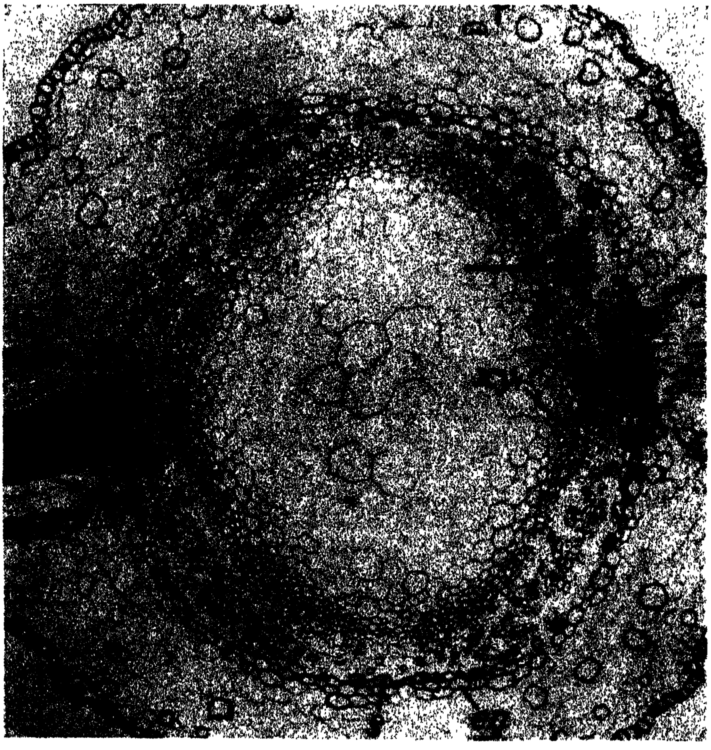

FIGURE 3.—Transection of a hypocotyl at a zone about 3.5 cm. above the base, at an early stage in secondary growth. The relation of the two lateral roots to the "paired" primary xylem strands (xy_1) is shown. An adventive primordium (indicated by arrow) lies beneath one of the four groups of fibers (*f*) (see fig. 8, *A*). Slight suberization in the layer of pericycle cells beneath the endodermis (*en*) is indicated at *s*. × 80.

may originate in pericycle tissue in a median position between the two protoxylem regions (fig. 4, *C*). The point of origin of this lateral root with reference to the xylem strands, which are alined tangentially at this level, indicates that the inherent potentiality of the pericycle cells over and between these protoxylem regions is an important factor in determining the initiation of primordia.

ADVENTIVE ROOT PRIMORDIA

At an early stage in secondary growth of the root, near the base of the hypocotyl adventive primordia are formed from a small group of cells in the cambium-phloem region slightly to one side of the protoxylem (fig. 5). No effort was made to determine whether or not pri-

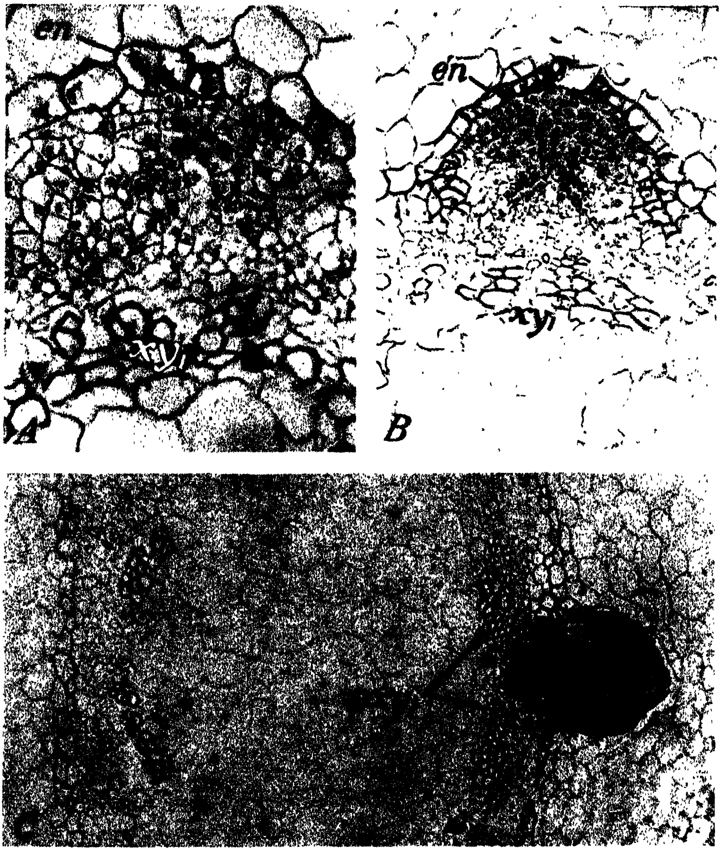

Figure 4.—*A* and *B*, Transections of lateral-root primordia at a zone near the base of the hypocotyl: *A*, Stage in which cell activity is evident over a comparatively large area, × 350; *B*, later stage characterized by rapid cell division with comparatively little cell enlargement, × 175. *en*, endodermis; xy_1, primary xylem region. *C*, Transection at the base of a hypocotyl showing position of origin of a lateral root with reference to the protoxylem regions (pxy_1) of the tangentially arranged primary xylem strands, × 90.

mordia developed in the root in other regions at either this or later stages.

In the hypocotyl adventive primordia are initiated in early stages of secondary growth and continue to be formed, with less frequency, however, in relatively late stages of secondary growth. At the early stages of secondary growth these primordia were observed (1) in the general region over the "paired" strands of primary xylem and (2) in the cambium-secondary-phloem region beneath the groups of fibers.

Progressive stages in the development of the primordia that originate in the general region over the "paired" primary xylem strands are shown in transverse sections in figure 6, *A*–*E*, and in longitudinal sections in figure 7, *A*–*D*. This region is characterized by a narrow zone of thin-walled meristematic cells that are presumably nondifferentiated derivatives of the pericycle. A cambium frequently develops in this region, but it does so comparatively late and after a considerable amount of xylem and phloem elements have been differentiated in each of the quadrants between the four "paired" primary xylem strands. Adventive primordia are initiated soon after the beginning of cambium activity in this region. The initiating cells are thin-walled and contain prominent nuclei, and most of them appear to be recent cambium derivatives. Growth and further development of the primordium up to the stage shown in figure 6, *E*, are largely the result of additions from the cambium, as can be observed in figure 6, *C* and *D*.

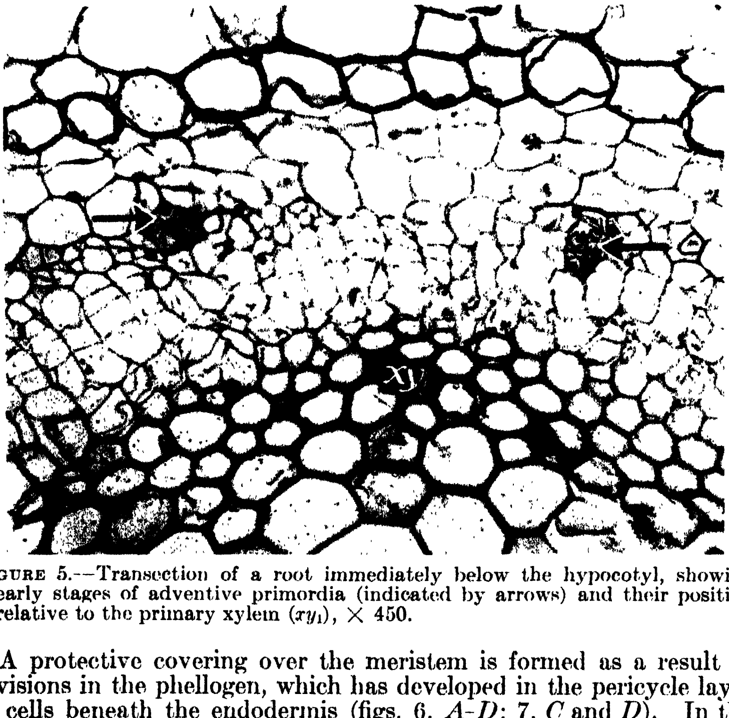

FIGURE 5.—Transection of a root immediately below the hypocotyl, showing early stages of adventive primordia (indicated by arrows) and their position relative to the primary xylem (xy_1), × 450.

A protective covering over the meristem is formed as a result of divisions in the phellogen, which has developed in the pericycle layer of cells beneath the endodermis (figs. 6, *A*–*D*; 7, *C* and *D*). In the areas over the young primordia the cell walls of these pericycle derivatives are thick and heavily stained. In this connection it is of interest to note that there is a tendency toward suberization in the outer layer of the pericycle over the primary xylem areas (fig. 3) at an early stage when the pericycle over the four arcs of fibers is still active in differentiating the pericycle fibers.

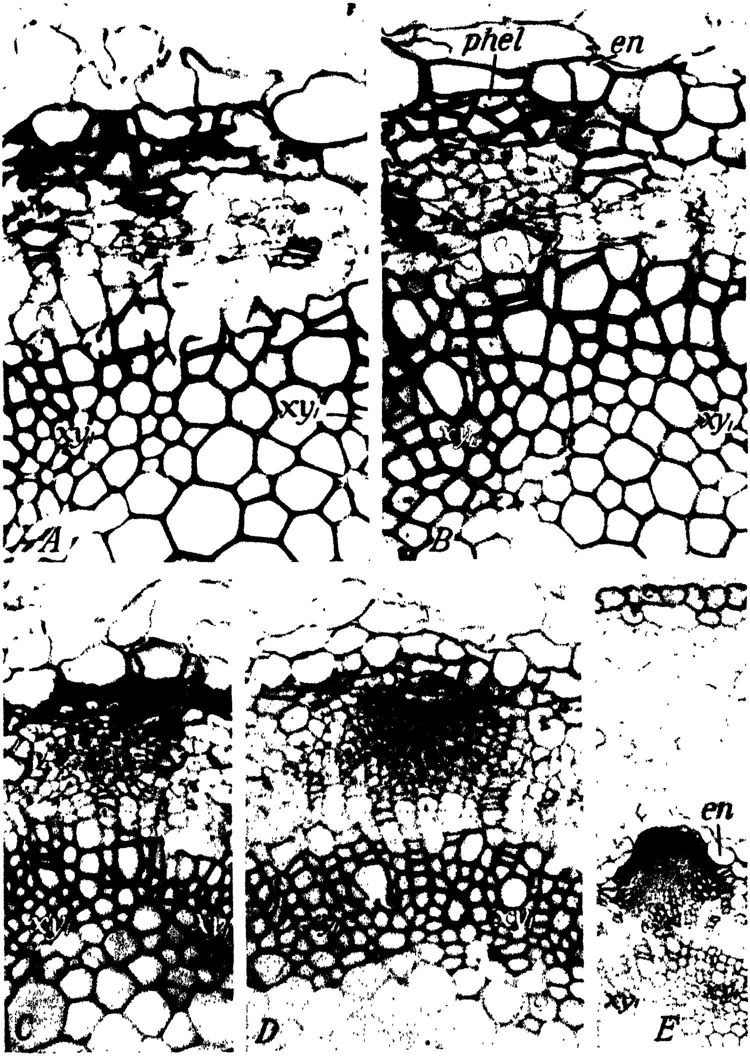

FIGURE 6.—Transections of hypocotyls showing the position of adventive primordia in the general region over the "paired" primary xylem strands and progressive stages in the development of primordia that have been initiated during early stages of secondary growth. xy_1, region of primary xylem; *en*, endodermis; *phel*, phellogen activity. *A* and *B*, × 400; *C* and *D*, × 225; *E*, × 110.

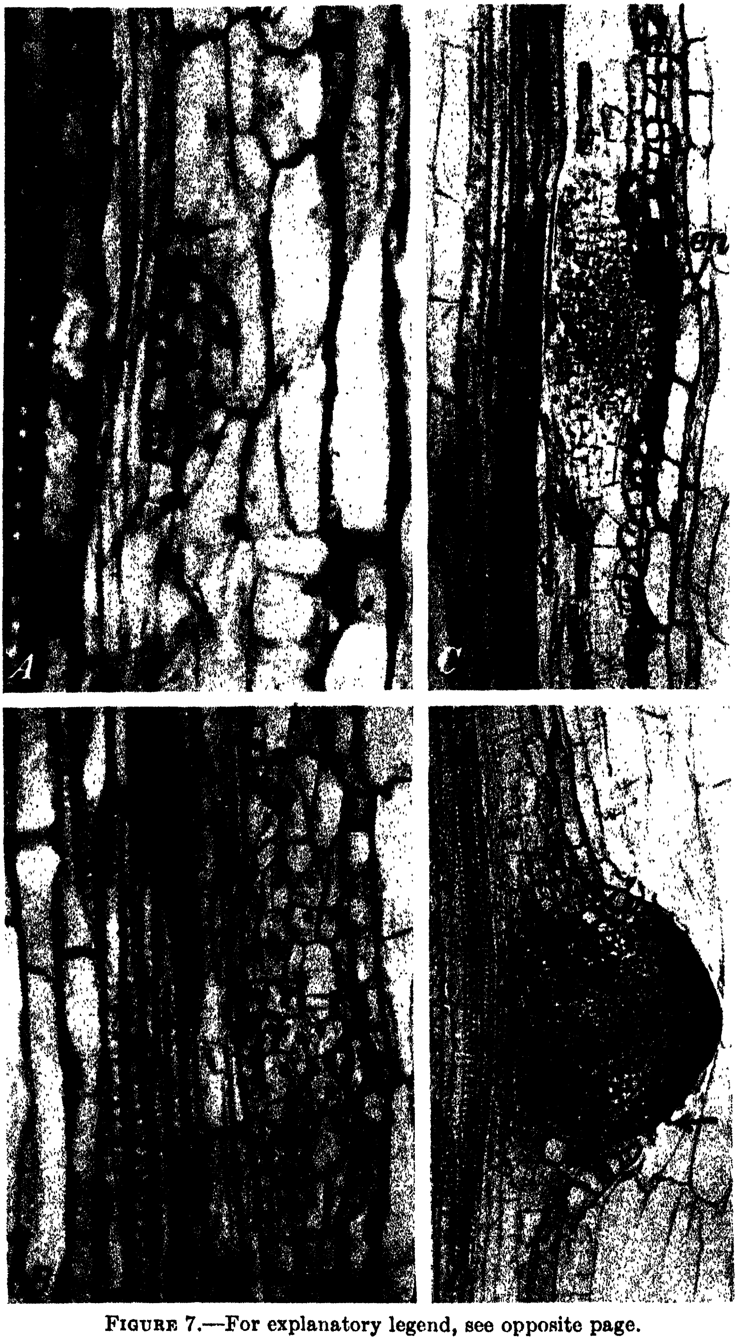

FIGURE 7.—For explanatory legend, see opposite page.

At the beginning of secondary growth, primordia are also initiated under these groups of fibers, as shown (by arrow) in figure 8, *A*, which is part of figure 3 enlarged, and in figure 8, *B*. A close series showing the progressive development of these primordia is shown in figure 8, *C–G*. Although the position of the large nucleated cell and the presence of the two nuclei in the cambium region beneath it suggest that the single large nucleated cell shown in figure 8, *C*, marks the initial stage of a primordium, positive identification is probably not warranted. In general, it is evident that most of these primordia are initiated in the cambium region from direct cambium derivatives. In only rare instances were there indications that the primordia originated from partially matured cells in the phloem (fig. 8, *D*). That the primordium may actually be "deep-seated" with respect to the present line of the cambium, because of intense meristematic activity with but little cell-wall delineation, is shown in figure 8, *B*.

With increasing vascular differentiation, activity becomes pronounced in the phellogen over the entire periphery. Relatively few primordia are formed after secondary growth has progressed much beyond the stage shown in figure 9, *A*, in which periderm is being formed. Those primordia that develop comparatively late originate in or near the phloem ray parenchyma and are usually initiated as a result of activity in a comparatively large group of cells (fig. 9, *A*, *B*, and *C*, *b*). Such cells lie in the general radial planes that pass through the primary xylem regions, in which planes lateral (fig. 9, *C*, *a*) and adventive roots (fig. 9, *C*, *c*) may have previously developed.

DISCUSSION AND CONCLUSIONS

The type of malformation discussed herein differs from that of infectious hairy root on apple and other hosts, in that these root formations occur over a general and indefinite region as compared with the more compact and localized masses of root primordia or of roots resulting from infection by the hairy root organism (*9*). Further study would be required to determine whether genetic or environmental factors are involved; limited observations indicate, however, that conditions favoring vigorous growth frequently may result in numerous roots on the hypocotyl of young seedlings. In the absence of information to the contrary, it is assumed that the economic losses due to discarding affected trees are relatively minor. The evidence, however, that the malformations described herein are nonpathogenic should aid nurserymen and inspectors in grading cherry trees.

In the anatomical studies an attempt was made to show the plan or pattern along which the hypocotyl develops as a result of activity of specialized tissue. Thus, four pairs of procambium strands in the upper levels of the transition zone become broadened tangentially in the older tissue at lower levels. At the base of the hypocotyl and in the adjacent root tissue the procambium forms an almost continuous sheath. The differentiation of the procambium into primary xylem

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FIGURE 7.—Radial sections of hypocotyls in vertical planes passing through the general region of the "paired" primary xylem strands, showing origin and development of adventive primordia. Phellogen activity is evident beneath the endodermis (*en*), and suberization of the cells forming a protective covering is pronounced. The arrow in *D* indicates remnants of cell walls of the collapsed endodermis. *A*, × 650; *B*, × 350; *C*, × 250; *D*, × 150.

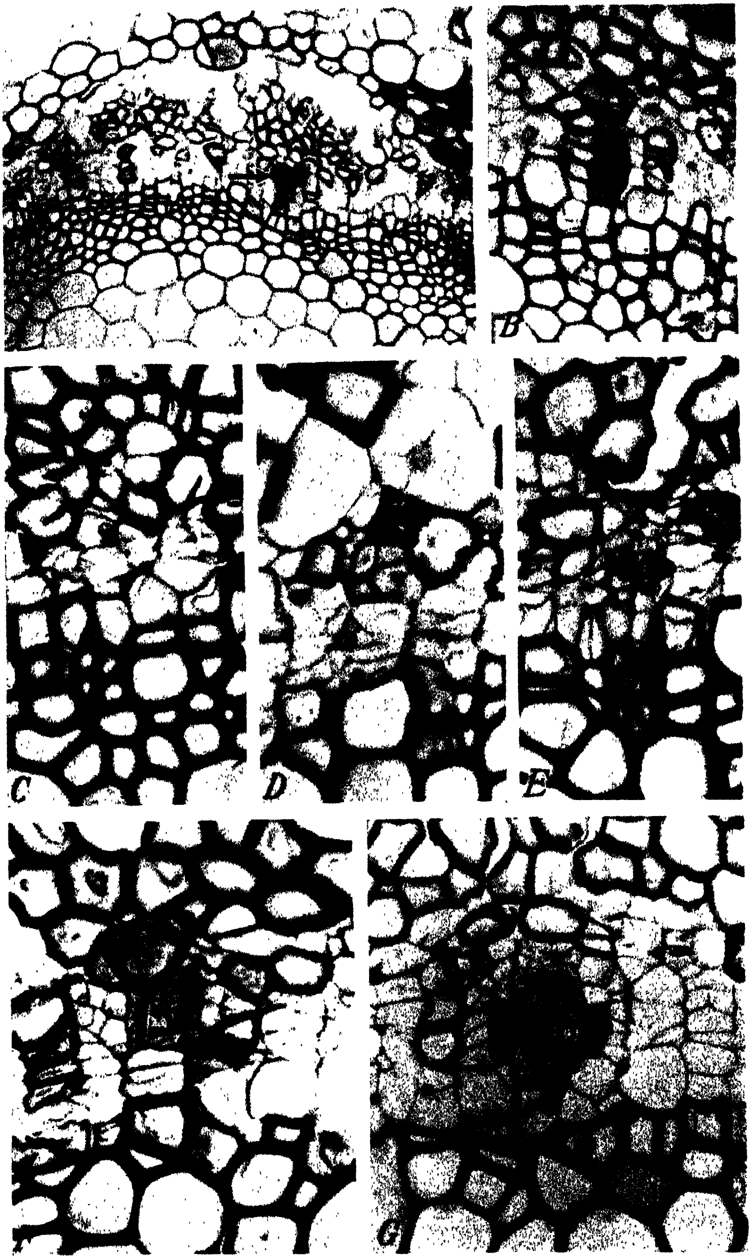

FIGURE 8.—Transections of hypocotyls showing (*A* and *B*) the adventive primordia that are initiated beneath the fibers (*f*) and (*C–G*) progressive stages in the development of such primordia. *A*, × 125; *B*, × 350; *C–G*, × 650.

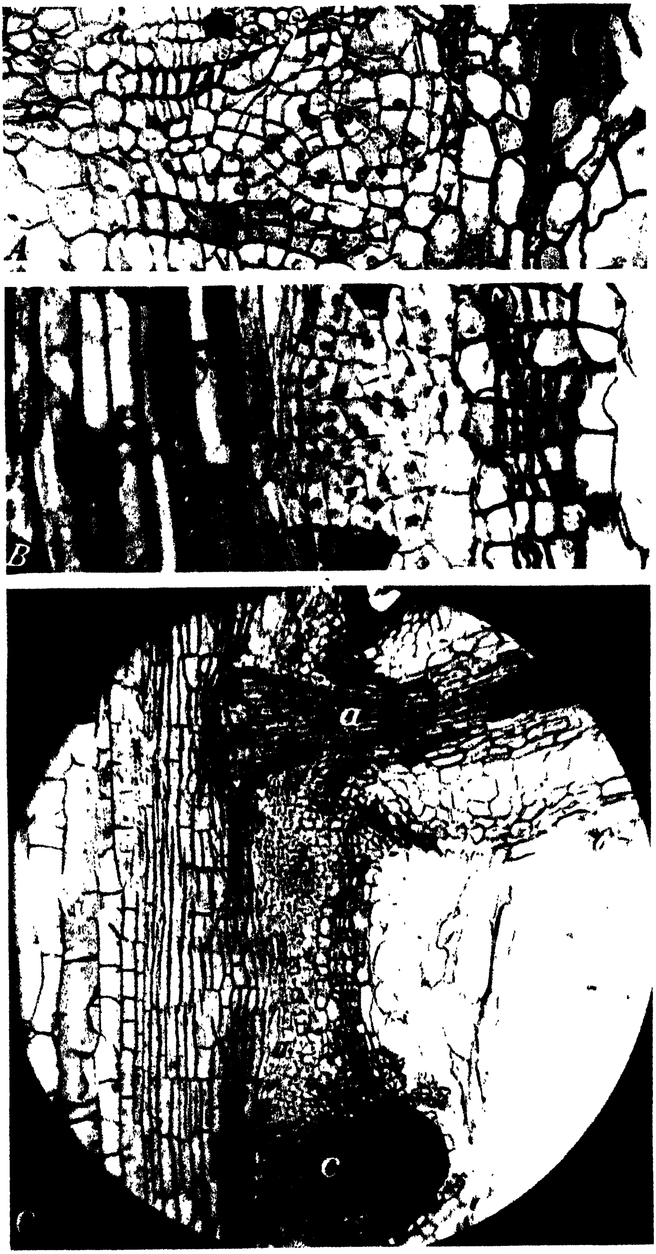

FIGURE 9.—Sections of hypocotyls showing origin of adventive primordia in relatively late stages of secondary growth: *A*, Transection, × 350; *B*, radial section, × 350; *C*, radial section, × 50. *a*, lateral root; *b* and *c*, adventive primordia.

elements therefore results in four broad arcs of xylem near the base of the hypocotyl, while nearer the upper level of the transition zone definite radial gaps are present in each pair of primary xylem strands. These "paired" primary xylem strands sometimes appear as widely separated entities (fig. 4, *C*), but more frequently the metaxylem elements of each of the "paired" strands tend to form a tangential line with the metaxylem elements of one strand from the adjacent "paired" strands. No attempt was made to determine an average for the length of the transition zone.

The vertical radial plane passing through the central regions of the broad primary xylem strands in the roots passes through the central region between each pair of primary xylem strands in the hypocotyl. Lateral root primordia are developed in the pericycle tissue in this plane. This area between and over the protoxylem strands is occupied by a meristem of pericycle which persists for a relatively long time; in other areas at the same level cambium and fibers have been differentiated. It can be reasoned that the inherent potentialities of these cells in which root primordia have their genesis are at least as important as the position of these cells in the organism.

At an early stage in secondary growth (fig. 3) the pericycle fibers are rapidly maturing. The bulge in the endodermis over each group of fibers presumably is the result of activity of pericycle cells in these regions; the pericycle cells over the four "paired" primary xylem strands remain as undifferentiated and comparatively inactive meristematic tissue. At this stage, no indication of a phellogen appears, but at a lower level, immediately below the root-stem junction, a phellogen is established by tangential divisions in the layer of cells of the pericycle underlying the endodermis very early in secondary growth. As is commonly observed, this makes for relatively early periderm formation and disintegration of the cortex in the root.

At the beginning of secondary growth adventive primordia are developed in meristematic tissue from cells that, like the pericycle cells over the primary xylem region, are relatively the least stabilized in differentiating other specialized tissue. Those primordia developing beneath the fibers are initiated in a cambium region from which relatively few xylem elements have been cut off; those primordia that develop in the strips of pericycle over the "paired" primary xylem regions are also initiated near or in a cambium region which has but recently been formed and in which ray parenchyma is common.

Relatively few primordia were formed in later stages of secondary growth after the periderm had become definitely established (fig. 9, *C*). These primordia were confined to a region in the broad phloem rays opposite the primary xylem region—a location in which numerous lateral and adventive roots occur earlier in the ontogeny of the hypocotyl. Relatively large numbers of partially matured cells appear to be involved in the initial stages of these primordia. These cells, however, are genetically related to a cambium, which is differentiating ray parenchyma only.

In almost all cases observed, the outermost cells of all the primordia abut on cells with thickened walls. Possibly such cells function as a mechanical barrier that prevents the loss of essential materials as has been suggested for the role of the endodermis (*7*); they may also serve to initiate meristematic activity as a result of pressure on the underlying meristematic cells. The slight tendency for suberization of pericycle cells over the primary xylem regions indicates at least that cell-wall thickening precedes the initiation of primorida, but it is recognized that pressure exerted by the growing meristem of the primordia might also produce the same effect.

SUMMARY

Pathological and anatomical studies were made to determine the nature of malformations characterized by an excessive number of roots on the mazzard cherry seedling rootstocks just below the ground line. In extreme cases these tufts of roots cause malformations so pronounced as to result in the discarding of the affected trees.

Although these root formations bear a slight resemblance to the hairy root disease of apple, caused by *Phytomonas rhizogenes* Riker et al., routine pathological studies have failed to disclose a causal organism. Inoculations on young mazzard seedlings with the hairy root organism resulted in infections that were typical of the hairy root disease but atypical of the root formations herein reported.

The anatomical studies demonstrated the presence of root primordia in the hypocotyl of young seedlings in sufficient numbers to account for the gross appearance, observed later, on nursery trees. The fact that many of these primordia bear such a definite morphological relationship to the parent tissue indicates that their presence is a normal occurrence in many seedlings.

Adventive primordia are formed in the hypocotyl most frequently at the beginning of or in the early stages of secondary growth. At this time they become differentiated (1) opposite the general regions of the primary xylem strands as a result of activity in a group of cells most of which appear to be cambium derivatives and (2) in the cambium region beneath the fibers as a result of activity of a small group of cells that are very recent cambium derivatives.

In later stages of secondary growth, adventive primordia are usually confined to the broad phloem rays opposite the primary xylem regions. They are differentiated as a result of activity of a comparatively large number of parenchyma cells.

The morphogenetic relation of the initiating cell or group of cells as well as the environment in which these cells develop has been briefly considered. This relation may be a factor in influencing the production of primordia. In general, adventive primordia are initiated in derivatives from those regions of the cambium that may be considered the least stabilized in producing xylem and phloem elements.

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EFFECTIVENESS AGAINST THE CALIFORNIA RED SCALE OF CUBE RESINS AND NICOTINE IN PETROLEUM SPRAY OIL[1]

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INTRODUCTION

The increasing difficulty of controlling the California red scale (*Aonidiella aurantii* (Mask.)) with hydrocyanic acid gas in parts of the citrus-producing area of California has brought about a serious problem in insect control (*9*).[2] Although oil emulsions are widely used in spraying citrus trees, it is difficult to secure a satisfactory kill of the scales on older wood with concentrations that are safe for the trees (*4*, *5*), particularly in heavy infestations.

In an effort to improve control practices, materials that might be expected to confer added toxicity to the sprays have been incorporated with the oils. Combinations of this kind have been employed in combating a number of other insects. Nicotine has been extensively used with oil.[3] Kagy and Richardson (*7*) showed that solutions of dinitro-o-cyclohexylphenol in oil were toxic to the San Jose scale (*Aspidiotus perniciosus* Comst.). L. H. Dawsey and the writer,[4] in work with mealybugs and the Florida red scale (*Chrysomphalus aonidum* (L.)) at New Orleans, La., found that the effectiveness of oil sprays was enhanced by the addition of rotenone, nicotine, or pyrethrum. In further work at Wooster, Ohio, with the Mexican mealybug (*Phenacoccus gossypii* Towns. and Ckll.) and the willow scurfy scale (*Chionaspis salicis-nigrae* Walsh), they showed that the addition of nicotine and rotenone increased the effectiveness of oil sprays. Although Smith (*10*) was unsuccessful in attempts to increase the toxicity of spray oils to the California red scale by the addition of various organic compounds, La Due (*8*) has reported that the addition of derris resins to oil with intermediary solvents gives increased toxicity to several scale insects of citrus.

The experiments reported herein include field tests of nicotine and cube resins with petroleum oil applied to the California red scale on lemon trees. The primary objectives were to determine whether the effectiveness of oil sprays could be improved by the addition of these two toxicants and, having demonstrated that point, to compare their relative effectiveness at selected concentrations.

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MATERIALS AND METHODS

All sprays contained a heavy mineral oil having the following specifications:

Saybolt viscosity at 100° F.	85 seconds.
Unsulfonatable residue	94.6 percent.
Specific gravity	0.858.
Percent distilling at 636° F.	5 percent.
Volatility (24 hours at 100° F.)	1.2 percent.

The cube resins contained 22.3 percent of rotenone and were completely soluble in carbon tetrachloride. Because of their limited solubility in petroleum oil, they were first dissolved in an intermediate solvent consisting of 1 part (by volume) of trichloroethylene and 2 parts of dibutyl phthalate. Ten parts of this solution were added to 90 parts of petroleum oil before emulsification, after which the resins were present partly in solution and partly in suspension in the oil.

Nicotine from a lot containing 95 percent of free base was used for the sprays containing this toxicant and was added to the spray mixture at the time of filling the tank.

To make the oil phase identical in all tests, the trichloroethylene-dibutyl phthalate mixture was always included in the oil phase, and all oil concentrations mentioned hereafter include 10 percent of this intermediate solvent. For example, a spray, with or without toxicant, that is said to contain 1 percent of oil included 0.9 percent of petroleum oil and 0.1 percent of the solvent mixture. Stock emulsions were prepared the day before spraying by agitating equal volumes of oil and an aqueous solution of ground bone glue with a motor stirrer for 20 minutes.

Spray applications were made with a small power sprayer, operated at 300 pounds' pressure, with a spray gun having a disk opening 3/32 inch in diameter. Trees were first sprayed from the inside and then circled on the outside from the ground. Tops of the trees were sprayed from a tower.

Oil deposits were determined according to the method described by Dawsey and Hiley (*3*) with the modification that the final drying was made at 55° C. while a slow stream of air was forced into the flasks. Each sample consisted of 80 or 100 disks 1.5 cm. in diameter, 4 from each of 20 or 25 leaves from a single tree. One or more samples were taken from each tree.

One series of tests was made in April and two series were made in October 1938. The experiments were conducted in an area near Whittier where the scale is considered to be resistant to fumigation (*9*).

The effectiveness of the sprays was determined by mortality counts of females in the late gray adult and older stages.[5] There were some survivors in the younger scales, an unknown proportion of which had advanced to the late gray or older stage when counted and which were included among the survivors; the young scales that had been killed were excluded. This method may have led to a low estimate of the mortality, but it would not change the order of ranking of the treatments.

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[5] It seems desirable to define the terms frequently applied to certain visible changes in the developmental periods of the California red scale. The early first stage designates the insects of both sexes from the time they emerge as crawlers until about midway in this stage when the body was become flattened and attached to the scale covering in preparation for molting. Scales in the remainder of the first stage, until this molt has been completed, are designated as in the first molt. In a similar way female scales in the second stage may be considered as in the early second stage and in the second molt, but in the case of the males this entire period is called the second stage. The males were not included in these tests. Female scales are called gray adults from the end of the second molt until fertilization takes place, and mature females following fertilization.

Ebeling (*6*) has shown that the immature stages of the red scale are more susceptible to oils than are the mature females, a finding that is in agreement with this writer's observations. Consequently it may be accepted that survival in the younger stages was considerably less than in the older stages examined in these experiments. The counts were purposely restricted to the more resistant stages.

Insects which had been attacked by parasites or predators, or which showed by their appearance that they were dead before the sprays were applied, were not included in the counts. They were listed separately, however, in counts of scales on the leaves and fruit for inclusion in estimates of population density. For the population-density determinations following the April applications the scales in the second molt and in the early gray stages were also included after adjustment for the smaller unit area covered by these stages.

In October the younger stages were less numerous on the fruit and leaves and were disregarded. The results for each leaf were recorded separately, and population density was expressed as number of scales per leaf. Fruits were marked off into quarters with ink lines, and the scales on each quarter lemon were recorded. The two axes of each lemon were measured, and an approximation of the area was obtained from the formula for the area of a prolate spheroid. Population density on the fruit was then expressed as number of scales per square centimeter.

Branches were divided into green wood and gray wood, the green wood being younger growth with a smoother surface. The wood was classified as lightly infested, heavily infested, or encrusted, according to whether less than one-fourth, one-fourth to three-fourths, or more than three-fourths of the surface was covered with scales. Encrusted green wood was not included in the mortality counts because of its scarcity. Wood in which there was considerable piling up of scales one over another was not examined.

EXPERIMENTS WITH SPRAYS CONTAINING 1 PERCENT OF OIL

Sprays containing 1 percent of oil were applied to infested lemon trees on April 21. One treatment consisted of oil alone, another of oil plus nicotine at the rate of 1 part of nicotine (100 percent) to 2,000 parts of dilute spray, and a third of oil plus cube resins at the rate of 1 part to 5,000 parts of dilute spray. The experimental trees were in 2 rows of 15 lemon trees each along the edge of a grove; 4 trees were treated with each material.

Since there were not sufficient scales on the leaves to give a reliable estimate of spray effects, mortality counts were limited to fruit and wood. The scales dead just before spraying, exclusive of those categories previously described—that is, insects attacked by parasites or predators or showing by their appearance that they were dead before the sprays were put on—averaged 1.9 percent on the fruit, 3.4 percent on green wood, and 5 percent on gray wood. There was no difference between lightly and heavily infested material. Natural mortality was not taken into account in determining spray effects on the fruit, but because of the low kill in heavy infestation on the gray wood, a correction for natural mortality was applied to the counts on wood.

Oil deposits were determined on samples consisting of 4 disks from each of 20 leaves per tree. Seven samples from each of the first two treatments were analyzed, 2 samples from 3 trees and 1 sample from the fourth tree; and 5 samples were taken from the oil-cube treatment. The average deposits are shown in table 1. The difference between the deposits from the oil-cube treatment and those from the other two treatments was small but was on the borderline of significance (*P* was about 0.05).

TABLE 1.—*Oil deposits and average mortality of California red scales on lemon fruits from sprays containing 1 percent of oil and the same concentration of oil plus nicotine or cube resins; sprays applied April 21, 1938*

Treatment	Oil deposit per square centimeter	Scales counted	Scales dead
	Micromilliliters	*Number*	*Percent*
Oil	47	1,547	61.3
Oil plus nicotine	48	1,160	90.4
Oil plus cube resins	56	1,200	93.0

There were few heavily infested fruits on these trees, and the insect counts were restricted to lemons having less than four scales per square centimeter of surface. The average mortality following the different treatments is shown in table 1.

A marked increase in the scale mortality on the fruits resulted from the addition of either nicotine or cube resins. Mortality in the trees sprayed with oil plus cube was only slightly higher than in the trees sprayed with oil plus nicotine.

Since it soon became evident that there had been no appreciable mortality among the females on the encrusted gray wood, counts were limited to the light and heavy infestations on green and gray wood. In most cases scales in each class of infestation on two to three twigs from each of the four trees were examined.[6] The results are summarized in table 2.

TABLE 2.—*Mortality of California red scales on lemon wood sprayed with a 1-percent oil spray and with an oil spray of the same concentration plus nicotine or cube resins, April 21, 1938*

Class of infestation	Oil		Oil plus nicotine		Oil plus cube resins		Average[1] mortality
	Total scales	Mortality	Total scales	Mortality	Total scales	Mortality	
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Percent*
On green wood:							
Light	566	43.4	558	47.8	665	80.1	57.1
Heavy	471	15.4	302	36.3	314	57.8	36.5
On gray wood:							
Light	708	27.0	545	38.0	454	62.6	42.5
Heavy	1,139	12.8	1,061	25.1	983	43.9	27.3
Average[1]		24.7		36.8		61.1	

[1] Each percentage has been given equal weight in estimating the average mortality. The number of scales examined in each class was not proportional to the population.

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[6] A statistical examination of data from another experiment showed that this number of twigs was sufficient to give a reliable estimate of the mortality when there was considerable survival of scales in all classes of infestation. When the mortality was very high, so that comparisons were based largely on the heavily infested gray wood, a larger number of twigs should be examined. Little accuracy was gained by increasing the number of scales counted in each class from 50 to 100 per twig when the number of twigs examined remained unchanged. It is probable that variations in the quantity of oil deposited or in the behavior of the oil film after spraying, due to unevenness of coverage or differences in the nature and texture of the bark, became more important than variations in susceptibility of the individual scales.

The data show the importance of the natural factors that affect control with toxicants in oils as well as the relative efficiency of the different treatments. These and other results leave no question about a differential response of the scale depending on the type of wood and the heaviness of the infestation. It seems probable that the gray wood absorbs more oil than the smoother green wood, and that consequently less oil reaches the insects on the older wood. The effect of density of infestation has been more accurately evaluated, and its probable causes have been discussed in publications dealing with other diaspine scales (*1*, *2*). The evidence therein indicated that population density also reduced the amount of oil acting upon the individual scales.

The addition of either nicotine or cube resins caused a significant increase in the mortality of scales on lemon wood, the effect of cube being especially marked.

None of these treatments caused any visible injury to the trees.

EXPERIMENTS WITH SPRAYS CONTAINING 2 AND 1.5 PERCENT OF OIL

In the next experiments, with sprays applied October 4, 1938, all concentrations were increased in an effort to determine whether commercial control of a heavy infestation of scale could be obtained by a single treatment, as well as to compare the efficiency of nicotine and cube resins when used with a higher percentage of oil. The oil concentration was increased to 2 percent, nicotine to 1 part in 1,500, and cube resins to 1 part in 4,000 parts of spray. The experimental trees were in the southwest corner of a lemon grove heavily infested with scales. Each treatment was applied to four trees.

Sprays were applied in another grove on October 20. While the general objectives were different from those in the other experiments described in this report, the same oil with and without cube resins was applied in two treatments, and these tests are comparable to the tests made October 4. Both sprays contained 1.5 percent of oil, and cube resins were added at the rate of 1 part to 4,000 parts of spray.

The natural mortality in the first grove averaged 0.8 percent on the fruit and 3 percent on the wood and in the second grove was about 5 percent on all parts of the trees. No corrections for natural mortality have been made in rating the different sprays.

Oil deposits were determined on samples of 4 disks taken from each of 25 leaves from each tree. Duplicate samples were taken from 5 of the 12 trees sprayed October 4 and from 6 of the 8 trees sprayed October 20. The average oil deposits are shown in table 3. The differences in the deposits were generally larger between trees than between duplicate samples from the same trees. Real differences in the average oil deposits on the trees within a plot were therefore indicated, although they were not such that any correlation between oil deposits and mortality within a given treatment could be detected.

There were no significant differences between the oil deposits in the two applications, even though different concentrations were used.

TABLE 3.—*Oil deposits from sprays containing 2 and 1.5 percent of oil, with and without the addition of nicotine or cube resins, applied to lemon trees October 1938*

Treatment	Date sprayed	Oil deposit per square centimeter
		Micromilliliters
2 percent of oil	Oct. 4	136
2 percent of oil plus nicotine	do	145
2 percent of oil plus cube resins	do	152
1.5 percent of oil	Oct. 20	141
1.5 percent of oil plus cube resins	do	147

No counts of scales on the leaves were made in the experiments of October 20. Mortality on the leaves sprayed October 4 is shown in table 4.

TABLE 4.—*Mortality of California red scales on lemon leaves resulting from sprays containing 2 percent of oil, with and without nicotine or cube resins, applied October 4, 1938*

Treatment	Total scales	Mortality
	Number	*Percent*
Oil	1,596	94.0
Oil plus nicotine	1,587	99.2
Oil plus cube resins	1,025	100.0

On the leaves sprayed with oil alone mortality ranged from 100 percent on the most lightly infested leaves to 84 percent on the leaves having 50 to 59 scales per leaf. When nicotine was combined with oil the kill was nearly complete, and when cube was added to the oil spray no survivors were found. No effect of population density was evident in the last 2 treatments. The effect of population density generally becomes difficult to demonstrate under conditions of very high mortality. It is possible that variations in mortality due to uneven coverage become more important and tend to obscure any density effects in the limited counts which can be made.

The mortality-population density curves for the scales on lemons sprayed October 4 are shown in figure 1.

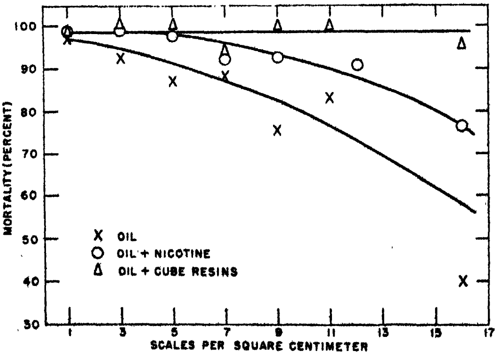

FIGURE 1.—Mortality of California red scales on lemons from sprays containing 2 percent of oil, with and without added nicotine or cube resins.

With both the oil alone and the oil plus nicotine the survival was greater at the higher levels of infestation, but after the oil-cube treatment no effect of population density was evident, the mortality being 98.3 percent in a count of 4,182 scales. The oil-cube was more effective than the oil-nicotine treatment, and both were superior to oil alone.

The fruit treated on October 20 was less heavily infested than that treated on October 4, the maximum density being 9 scales per square centimeter. A trend toward lower mortality at the higher densities was evident among the scales sprayed with oil alone but not among those sprayed with oil plus cube. A regression line fitted to the results of the first treatment showed that the survival increased, on an average, about 2 percent with each increase of 1 scale per square centimeter. The average mortality among the scales sprayed with oil alone was 88 percent, in those sprayed with oil plus cube, 95 percent. Of the scales surviving the oil-cube treatment 48 were on a quarter of one lemon bearing 71 scales. If they are excluded, the average mortality from this treatment was about 97 percent.

Scales in the five classes of infestation—light and heavy on both green and gray wood, and encrusted on gray wood—were examined after being sprayed with 2 percent of oil. Mortality of scales in each class on three or four twigs from each of the four trees in a plot was determined. Since there was not sufficient encrusted material on the trees sprayed with 1.5 percent of oil to give a reliable estimate of the mortality, counts were restricted to the first four classes of infestation, and from four to six twigs per tree were examined. The results are summarized in table 5.

TABLE 5.—*Effectiveness of sprays containing 2 and 1.5 percent of oil, alone and with nicotine or cube resins, against California red scales on lemon wood, October 1938*

Treatment	Green wood				Gray wood						Average mortality
	Light infestation		Heavy infestation		Light infestation		Heavy infestation		Encrusted		
	Total scales	Mortality	Total scales	Mortality	Total scales	Mortality	Total scales	Mortality	Total scales	Mortality	
2 percent of oil (Oct. 4):	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Percent*
Oil alone	832	89.8	691	80.0	572	81.5	778	58.6	577	45.4	71.1
Oil plus nicotine	656	98.2	700	91.4	565	97.7	778	87.5	599	62.9	87.5
Oil plus cube	608	100.0	655	98.8	549	97.8	547	94.6	1,096	88.8	96.0
1.5 percent of oil (Oct. 20):											
Oil alone	718	88.0	767	77.2	650	78.5	1,542	58.1	-----	-----	75.5
Oil plus cube	504	99.6	782	95.8	673	95.4	2,240	91.2	-----	-----	95.5

Both nicotine and cube resins caused a marked increase in mortality, but the results from the oil-cube treatment were especially striking. As in experiments with sprays containing 1 percent of oil, more scales survived in the heavy than in the light infestations, and more on the gray than on the green wood.

As a result of the heavy infestation of scales in the grove sprayed on October 4, many of the leaves and fruits were dropping before the sprays were applied. This process appeared to be accelerated by the treatments, but the amount of defoliation and fruit drop seemed to depend on the degree of infestation and condition of the trees rather than on the materials applied. A similar condition prevailed in the rest of the grove, which was sprayed by a commercial operator. There was no evidence of injury due to the toxicants added to the oil. The grove sprayed on October 20 was less heavily infested, and no plant reaction to the sprays was observed.

COMPARISON WITH COMMERCIAL TREATMENT

The remainder of the grove containing the trees sprayed on October 4 was sprayed on October 2 by a commercial operator with a 1.67-percent concentration of a heavy medium-emulsive oil. The mortality was comparable to that obtained with the 2-percent oil spray in the experimental plot. The following August, when the entire grove was sprayed again, the infestation on the wood of the experimental trees that had been sprayed with oil plus cube or with oil plus nicotine was much lighter than the infestation on the trees that had been given the commercial treatment without added toxicant. The fruit on these trees appeared commercially clean, whereas many heavily infested fruits were found in the rest of the grove. Fruits on the trees sprayed with 2 percent of heavy oil were intermediate as to degree of infestation.

DISCUSSION

The oil deposits from the sprays applied in October were greater than would be expected from the increase in oil concentration over that used in April. In general, it has been found that the rate of increase in oil deposits declines with increasing oil concentrations; yet the deposits from the 1.5- and 2-percent oil sprays were more than double those from the 1-percent sprays. While this result may have been due to differences in the emulsions, differences in the texture of the leaves, dust or sooty mold on the trees, and abrasion by red spiders may have been contributing factors. It is well known that the amount of oil deposited cannot always be controlled by the concentration of oil applied.

Two features of the insecticidal results are of special interest, namely, the low mortality of scales on the heavily infested older wood resulting from sprays of oil alone and the effectiveness of cube resins in oil in this type of infestation. In the experiments in which 1 percent of oil was used scale mortality on the heavily infested gray wood was only 12.8 percent, whereas in the experiments in which 1.5 and 2 percent of oil were used mortality was 58.1 and 58.6 percent, respectively. While both nicotine and cube resins, when added to the oil, produced significant increases in mortality, the best kills were obtained with oil plus cube, the mortality being 43.9, 91.2, and 94.5 percent, respectively, from the three series of sprays. Although the counts were restricted to the less susceptible stages, and the residual effect of the oil film against the active crawlers has not been considered, the low mortality in the first experiments does not favor the possibility of obtaining a satisfactory kill in heavy infestations with oil concentrations materially lower than those in present use. Nevertheless, the prospect of securing better control by the addition of toxicants to oils at the concentrations now generally applied is encouraging.

Whether the increased cost of adding a toxicant to the oil would be justified by the lower scale populations or by a necessity for less frequent treatments requires further investigation, including tests on a larger scale and observations on the course of reinfestation extending over a longer time. The value of combinations of toxicants with the lighter oils, which are considered to be less injurious to citrus, is also being investigated.

SUMMARY

Experiments were made to test the relative effectiveness against the California red scale (*Aonidiella aurantii* (Mask.)) of sprays of a heavy petroleum oil, oil plus nicotine, and oil plus cube resins.

In the first experiment all sprays contained 1 percent of oil. Nicotine was used at the rate of 1 part to 2,000 parts, and cube resins at 1 part to 5,000 parts of spray. The cube resins contained 22.3 percent of rotenone. Later experiments included sprays with 2 and 1.5 percent of oil, to some of which nicotine was added at the rate of 1 to 1,500, and to others cube at 1 to 4,000. The cube resins were dissolved in an intermediary solvent consisting of 1 part of trichloroethylene and 2 parts of dibutyl phthalate, and this solvent was included in all the sprays.

Mortality determinations were made only on the females in the gray adult and older stages. Mortality in the more susceptible younger scales and the residual effect of the oil film upon the crawlers were not considered. Counts were made separately on the leaves, fruit, and wood.

Both nicotine and cube in oil gave marked increases in the mortality of scales on all parts of the tree as compared with oil alone. The sprays containing cube resins were more effective against the scales on the wood than those containing nicotine, and in the treatments in which 2 percent of oil was used they were more effective on all parts of the tree. In heavy infestations on older wood the addition of cube resins increased the mortality from 12.8, 58.1, and 58.6 percent to 43.9, 91.2, and 94.5 percent for the applications of 1, 1.5, and 2 percent of oil, respectively.

Spray mortality varied inversely with the density of infestation, except in cases of very high mortality, where a population density effect was not always evident. Survival was highest in the heavily infested gray wood.

There was no evidence of injury to the trees due to the added toxicants.

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DIFFERENTIATION OF THE TWO GENETIC FACTORS FOR RESISTANCE TO THE HESSIAN FLY IN DAWSON WHEAT[1]

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SUMMARY

Investigations on the resistance of Dawson wheat to the hessian fly are reported. These studies deal with material derived from the crosses Dawson × Poso and Dawson × Big Club and advanced toward the commercial type by different numbers of backcrosses. Three backcrosses, selections having the genetic constitution $H_1H_1H_2H_2$, $H_1H_1h_2h_2$, and $h_1h_1H_2H_2$ were isolated, and their performance during several years was recorded. They were then recombined and the progeny cataloged for hessian fly reaction in F_2 and F_3 generations. In addition to confirming the presence of two factors for resistance to the hessian fly, the data demonstrate the successful isolation, differentiation, and recombination of the two Dawson factors. They further demonstrate the successful determination of these resistance factors in two additional backcrosses during a time of relatively low infestation. Finally, the performance of the experimental variety, Big Club 38, over a 4-year period demonstrates the successful application of the breeding method for control of the hessian fly.

INTRODUCTION

In 1936 Cartwright and Wiebe[3] reported that the wheat variety Dawson contributed two genetic factors for resistance to the hessian fly (*Phytophaga destructor* (Say)) found in California, and mentioned a backcrossing program designed to transfer this resistance to the susceptible commercial varieties Poso and Big Club. Two years later Briggs[4] indicated progress on the breeding phase of this program. Since the writers are now engaged in a rather comprehensive survey of genetic factors for resistance to the hessian fly, it seems timely to present data to confirm further the presence of two genetic factors in the variety Dawson and to report their isolation and differentiation.

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[1] Received for publication December 12, 1942. This work was carried out in cooperation with the Department of Agronomy, University of California.

[2] The writers are indebted to L. G. Jones, W. B. Cartwright, and F. N. Briggs for helpful suggestions and criticisms in the conduct of this work and preparation of the manuscript.

[3] CARTWRIGHT, W. B., and WIEBE, G. A. INHERITANCE OF RESISTANCE TO THE HESSIAN FLY IN THE WHEAT CROSSES DAWSON X POSO AND DAWSON X BIG CLUB. Jour. Agr. Res. 52: 691-695, illus. 1936.

[4] BRIGGS, F. N. THE USE OF THE BACKCROSS IN CROP IMPROVEMENT. Amer. Nat. 72: 285-292. 1938.

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MATERIALS AND METHODS

The breeding stocks used in the isolation and differentiation of the Dawson factors were derived from the crosses made in 1931 and studied by Cartwright and Wiebe. They have been grown and tested on a fixed backcross breeding schedule in the vicinity of Birds Landing, Calif. The reaction of the parent varieties to fly infestation has been consistent in all the years that they have been tested. Their performance for the years 1935 to 1941 is shown in table 1. In this table are also recorded, for comparison, the infestations obtained in the experimental variety Big Club 38 from 1938 to 1941. This variety is composed of F_3 lines selected in 1937 from the third backcross of Dawson × Big Club to Big Club, and its behavior during the 4 years that it has been under observation bespeaks general success for the breeding program.

TABLE 1.—*Hessian fly infestations in parent varieties and 1 derived variety of wheat at Birds Landing, Calif., 1935–41*

Variety	Percentage of plants infested						
	1935	1936	1937	1938	1939	1940	1941
Dawson	2	0	0	0	0	2	2
Poso	98	83	94	76	52	67	85
Big Club	98	95	93	84	68	69	87
Big Club 38	----	------	------	0	0	4	2

In breeding wheats for fly resistance, if the genetic factors have been properly recovered in consecutive backcrosses to the susceptible parents, each successive population of plant families should have a frequency distribution of fly infestations comparable to that obtained in the original cross. Likewise in cases such as this, where two genetic factors for resistance are involved, it should be possible to identify definitely the postulated two factors for resistance. To do this, each factor must be isolated in a line by itself so that its monofactorial inheritance can be established. In this work the two factors were isolated by backcrossing F_1 plants of the cross Dawson ($H_1H_1H_2H_2$) × Poso ($h_1h_1h_2h_2$) to Poso, as schematically shown below.

Cross: $H_1H_1H_2H_2 \times h_1h_1h_2h_2$
F_1: $H_1h_1H_2h_2$
Backcross: $H_1h_1H_2h_2 \times h_1h_1h_2h_2$
F_1's: $H_1h_1H_2h_2$
$H_1h_1h_2h_2$
$h_1h_1H_2h_2$
$h_1h_1h_2h_2$

The F_1's of the backcross segregate in the proportion 1 : 1 : 1 : 1; that is, one-fourth of the plants possess both of the Dawson factors for resistance, one-fourth possess one of them, one-fourth possess the other, and one-fourth possess neither.

In these studies plants were classified as infested or uninfested depending upon whether or not puparia were present. The number of plants examined from each row varied considerably but was never less than 25. Checks of the susceptible commercial varieties Poso and Big Club were grown alternately in each eleventh row throughout the nurseries, and where possible the nursery stock was grown in proximity to infested stubble. Infestations in the vicinity of Birds Landing vary less from year to year than in most areas, but unfortunately they were at a rather low level during the years of these experiments. From 1938 to 1940 infestations were not heavy enough for precise genetic analysis, but by interpolation of the data on pedigreed hybrid lines sufficient information has been assembled for this purpose.

EXPERIMENTAL RESULTS AND INTERPRETATION

The data of Cartwright and Wiebe showed that an F_2 family derived from Dawson × Poso ($H_1H_1H_2H_2 \times h_1h_1h_2h_2$) was 13.7 percent infested by the hessian fly. The data obtained on 40 F_2 families in 1936 showed that this value might range from 5 to 25 percent in small-sample analyses, with most of the families falling below a midclass value of 17.5 percent. The tests of 188 families in the same year indicated that the F_2 families obtained by backcrossing an F_1 plant to susceptible Poso fell within the expected proportion of one-fourth susceptible, one-half segregating for single factors, and one-fourth segregating for two factors. Since the distribution of the two-factor lines was determined by Cartwright and Wiebe, the behavior of these families was not extensively studied, but that of 100 F_3 families derived from $H_1h_1H_2h_2$ was in agreement with the two-factor hypothesis regarding Dawson.

Since the major objective of the research was to identify the two factors of Dawson, the single-factor segregating families of 1936 were tested in F_3 rows in 1937. Of 124 families tested 29 were susceptible, which was close to the 31 theoretically expected. These data, along with the distributions of the F_2 families mentioned above, are given in table 2.

In order to advance the breeding program by utilizing the backcross principle and testing the genetic concept upon which it was based, certain lines derived from the F_3 tests of 1937 were selected for further study. The genetic constitution and reactions to the hessian fly for these lines are shown in table 3. In distinguishing between H_1 and H_2 factors for resistance, the initial differentiation was based on an F_2 infestation range of 25 to 42 percent. Subsequent tests have indicated that H_1 and H_2 are about equal in their ability to impart resistance, and definitely inferior to the double combination as it occurs in lines 6078 and 6110 (table 3) or in the variety Dawson itself. In other words, a wheat having the factor H_1 or H_2 alone would show a range of 0 to 10 percent infestation over a term of years, in contrast to the reaction of Dawson shown in table 1.

TABLE 2.—*Distribution of F_2 and F_3 2-factor and 1-factor families of the third backcross of Dawson × Poso to Poso, at Birds Landing, Calif., in 1936 and 1937* [1]

Genetic constitution	Number of rows in indicated class (percentage) of hessian fly infestation																				Total
	2.5	7.5	12.5	17.5	22.5	27.5	32.5	37.5	42.5	47.5	52.5	57.5	62.5	67.5	72.5	77.5	82.5	86.5	92.5	97.5	
F_2 (1936):																					
$H_1h_1H_2h_2$	14	8	10	6	2																40
$H_1h_1H_2h_2 \times h_1h_1h_2h_2$	6	14	12	18	4	20	10	19	12	14	7	3	5	1	0	1	4	5	9	24	188
F_3 (1937):																					
$H_1h_1h_2h_2$ or $h_1h_1H_2h_2$	26	6	6	7	8	6	8	9	2	3	5	6	2	1	0	0	1	2	7	19	124
$H_1H_1H_2H_2 \times h_1h_1h_2h_2$	45	11	16	6	1	1	3	3	3	3	2	1	0	0	0	1	0	0	2	2	100

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[1] Poso was 83 percent infested in 1936 and 94 percent in 1937.

TABLE 3.—*Hessian fly infestations of selected lines of the third backcross of Dawson × Poso to Poso and of their backcross progeny, at Birds Landing, Calif., 1936–41*

Selection	Genetic constitution	Percentage of plants infested						
		Third backcross					Fourth back-cross	Fifth back-cross
		F_2 1936	F_3 1937	F_3 1939	F_3 1940	F_3 1941	F_2 1938	F_2 1939
6078	$H_1H_1H_2H_2$	8	0	0	0		5	3
6110	do	8	0	0	0		7	5
6179	$H_1H_1h_2h_2$	25	0	0	8	12	13	15
6194	do	28	3	0	4		18	10
6232	$h_1h_1H_2H_2$	42	3				17	10
6270	do	29	6	0	4	8	22	10

A number of recombinations between the several extracted lines were studied in F_2, as shown in table 4. Although these studies were made in different years, they contribute little in themselves because of the low level of infestation.

TABLE 4.—*Hessian fly infestations of F_2 generation hybrids between selections from the third backcross of Dawson × Poso to Poso, at Birds Landing, Calif., in 1938 and 1940*

Selections crossed	Genetic constitution	1938		1940	
		Plants observed	Plants infested	Plants observed	Plants infested
		Number	*Percent*	*Number*	*Percent*
6078 × 6179	$H_1H_1H_2H_2 \times H_1H_1h_2h_2$	284	5	130	2
6110 × 6270	$H_1H_1H_2H_2 \times h_1h_1H_2H_2$	242	3	150	0
6179 × 6270	$H_1H_1h_2h_2 \times h_1h_1H_2H_2$	492	6	204	3
6194 × 6232	$H_1H_1h_2h_2 \times h_1h_1H_2H_2$	497	7	260	2

Studies of the F_3 generation were made in 1940, as shown in table 5. Here, again, low infestations were troublesome, as indicated by the extreme dispersal of the susceptible Poso parent with some rows showing only 30 percent of infested plants. Confirmation of the previously assigned genetic constitution of all extracted selections from the third backcross of Dawson×Poso to Poso is also given in table 5. In the crosses between two-factor and single-factor selections no susceptible families were recovered, whereas in crosses between two single-factor lines heterozygous and susceptible families were recovered. Supporting these data are the distributions observed for certain of the selections successfully backcrossed twice to Poso, in which the proportion of resistant rows is much higher for the two-factor lines.

TABLE 5.—*Distribution of parents and F_3 rows of hybrids between selections from the third backcross of Dawson × Poso to Poso and of selections further backcrossed to Poso, at Birds Landing, Calif., in 1940*

Group and pedigree	Number of rows in indicated class (percentage) of hessian fly infestation																			Total
	2.5	7.5	12.5	17.5	22.5	27.5	32.5	37.5	42.5	47.5	52.5	57.5	62.5	67.5	72.5	77.5	82.5	87.5	92.5	
Parent varieties:																				
Dawson	2																			2
Poso							3	0	4	1	0	3	4	4	6	3	2	2	2	34
Selection backcrosses:																				
6078×Poso ^{2} [a]	10	7	2	1																20
6110×Poso ^{2}	9	3	1	2	1	3	1													20
6194×Poso ^{2}	2	2	1	5	2	0	0	2	0	1	0	2	0	0	1	0	1			19
6232×Poso ^{2}			1	0	0	1	0	2	2	0	0	0	2	0	0	0	2			10
6270×Poso ^{2}	4	2	0	2	3	0	0	3	0	0	0	1	0	2	0	2	1			20
Selections crossed:																				
6078×6179	26	3																		29
6110×6270	26	1	2	0	1															30
6179×6270	48	5	1	3	1	0	0	1												59
6194×6232	32	8	1	5	1	1	1	2	0	0	0	0	0	0	0	0	1			52

[a] The superscript "2" indicates that the selections were backcrossed twice to Poso.

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FOOD RESERVES AND THEIR TRANSLOCATION TO THE CROWN BUDS AS RELATED TO COLD AND DROUGHT RESISTANCE IN ALFALFA[1]

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INTRODUCTION

The physiological role of food reserves in many crop plants has been studied and reported to be a factor in the hardening process of plants to different adverse conditions. Time-of-cutting experiments with alfalfa (*Medicago sativa* L.), conducted by the Division of Forage Crops and Diseases in cooperation with the Kansas Agricultural Experiment Station, at Manhattan, Kans., during the last 35 years, have demonstrated the effects of different cutting treatments on hay quality, on longevity of stands, and on the storage of carbohydrates and nitrogen in the roots.

The study reported herein was made to determine the importance of food reserves in relation to the physiological processes that occur during the hardening of alfalfa to adverse conditions. This study was designed particularly to show the effect of time of fall cutting on the development of the crown buds and the effect of stored organic food reserves in the roots on their cold resistance. As used in this paper, the term "crown buds" is defined as the new growth, either pink or white in color, originating from the crown or crown shoots under the surface of the soil. As soon as a bud developed a green tip it was no longer classified as a crown bud.

REVIEW OF LITERATURE

Nelson (*17*)[3] reported that a reduction of vigor and productivity accompanied a lowering of food reserves in alfalfa roots, and other investigators (*5*, *11*) have obtained similar results. The present writer (*7*) found that the critical period in the life of alfalfa is in the fall and that removal of the late summer growth early in the fall lowered the carbohydrate and nitrogen content of the roots to such an extent as to make the plants more susceptible to winter injury. Rather and Dorrance (*21*) reported that grazing in the fall reduced the dry matter in the roots and the number and vigor of crown buds. Electrical conductivity tests of the roots indicated that alfalfa ungrazed after August 28 hardened off much better.

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[2] The author gratefully acknowledges his indebtedness to H. L. Westover, of the Division of Forage Crops and Diseases, for aid in planning the experiments and for valuable suggestions and criticisms in the preparation of this paper; and to E. J. Kreizinger, M. L. Peterson, and George V. Goodding, also of this Division, for assistance during the course of the experiment.

[3] Italic numbers in parentheses refer to Literature Cited, p. 45.

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Silkett et al. (*24*) concluded that alfalfa plants cut in September develop fewer crown buds per plant than those cut earlier or during October. Johnsson (*12*) found that higher yields were associated partly with a larger amount of reserve food stored in the roots and partly with a greater number of winter-hardy crown buds.

Ireland (*10*) noted a difference in fluctuations of the cell-sap concentration during the winter in hardy and nonhardy varieties of alfalfa. Dexter (*2*) stated that "hardening of plants is favored by conditions which tend toward the accumulation or conservation of carbohydrates and other reserve foods." Kneen and Blish (*13*) showed a positive correlation between cold resistance and sucrose content in winter wheat plants. The writer (*7*) found that conditions of top growth favorable for high food storage in alfalfa can be brought about by proper cutting practices.

The work of other investigators has established that, coincidental with the increase in drought resistance in some plants, there is a pronounced increase in some carbohydrates and a decrease in others. Lvoff and Fichtenholz (*14*) and Vasiliev and Vasiliev (*26*) noted that following wilting there was an increase in the hydrolysis of starch and an increase in sugars.

These experiments, as well as others, suggest that the hardening processes to cold and to drought are similar and may be controlled to some extent in alfalfa by proper cutting practices.

Dunn (*4*) demonstrated that the protoplasmic constituents of the cells of alfalfa are of primary importance in the hardening to cold. Newton and Martin (*20*) found that the amino nitrogen content of the wheat plant increased during the hardening process and that high carbohydrate and nitrogen content are necessary to cold resistance. Harvey (*9*) held that the splitting of protein into less easily precipitated forms is a method of adaptation of plants to cold. Mark (*15*), working with alfalfa, found a marked increase in the content of both protein and nonprotein nitrogen from uncut plants while that of continuously cut plants remained about constant, indicating that, if a high content of protein nitrogen is related to cold resistance, there is a distinct difference in favor of uncut plants. Greathouse and Stuart (*8*) concluded that a high concentration of total sugars and total nitrogen was associated with cold resistance. Tysdal (*25*) reported that the concentration of sugar and amino acids, as found in the extract of alfalfa roots, influenced the protective activity. Miller (*16*), in a discussion of synthesis of proteins by green plants, stated that proteins are not formed in the plant without a supply of carbohydrates. Thus there probably is a close association of the stored food reserve and the ability of a plant to harden off. Dexter et al. (*3*) measured this hardening by electrolysis, and Newton and Brown (*18*) measured it by determining the bound water, stating that hydrophilic colloids bind water and increase the concentration of aqueous solutions and that factors affecting drought resistance include those concerned in absorption, transpiration, and wilt endurance. Newton et al. (*19*) also stated that moisture content is less in hardy varieties and that the resulting concentration of colloids and sugars in all fluids increases the resistance to freezing.

From this brief review of literature, it is evident that a better understanding of the carbohydrate metabolism and translocation within the alfalfa plant is necessary to correlate it with the hardening processes and controlled cutting practices.

METHODS OF EXPERIMENTATION

OUTLINE OF EXPERIMENTS

The experiments were designed to show the effect of fall cuttings on crown-bud development and to correlate these cuttings with the food reserve storage and cold resistance of the roots and buds.

In earlier tests the writer (*6*, *7*) showed that fall cuttings of alfalfa more than any other affected the ability of the less winter-hardy varieties to maintain stands and also that these cuttings exerted a decided effect on the yield of hay from the first cutting of the following season.

In 1935 an experiment was begun in which cuttings were made from three replications, as shown in table 1. Particular attention was given to the effect of the number of days in the fall growing period. The later time-of-cutting experiments mentioned in the report follow this outline, except that the first three cuttings were all made at the same time and by regulating the time of the fourth and fifth cuttings various lengths of fall growing periods were obtained. Data on the crown buds and roots were taken on 100 plants dug from each plot. The following determinations were made and recorded: For crown buds, number, length, dry weight, percentage of carbohydrates and of total nitrogen; for roots, percentage of carbohydrates and of total nitrogen.

TABLE 1.—*Outline of experiments showing summer treatment by cuttings, date of last cutting, and fall treatment*

Plot No.	Summer treatment by cuttings					Date of last cutting	Fall treatment
	1	2	3	4	5		
1	[1] B	B	B	B	B	Oct. 8	Cut Oct. 8.
	B	B	B	B		Aug. 26	Uncut.
2	B	B	B	B		do	Cut Oct. 8.
	B	B	B	B		do	Uncut.
3	B	B	B	[2] FB		Sept. 4	Cut Oct. 8.
	B	B	B	FB		do	Uncut.
4	B	B	FB	FB		Sept. 14	Cut Oct. 8.
	B	B	FB	FB		do	Uncut.
5	B	FB	FB	FB		Sept. 28	Cut Oct. 8.
	B	FB	FB	FB		do	Uncut.

[1] B = bud.

[2] FB = full bloom.

In 1937 a new experiment was started on a field previously seeded to Kansas Common and Ladak alfalfa. The object of this experiment was to verify the conclusions reached in the earlier work and to determine whether there was a varietal difference in the development of resistance to cold. In addition to the data taken as in the previous experiment, determinations of bound water, specific conductance of exosmosed material, total water, and dry weight were made on the crown-bud material.

FIELD SAMPLING

All the top growth was removed from the root samples and discarded, the new crown buds were picked from the crowns, and the roots were trimmed to 8-inch lengths, including the closely clipped crown. The fresh crown-bud material was used for determinations of bound water and specific conductance,extreme care being taken to minimize the loss of water while the samples were being prepared. That part of each sample to be used for chemical analysis was immediately oven-dried at 98° C. The individual pieces of buds were small, allowing rapid heat penetration and thus stopping all enzymatic action. The root samples for chemical analyses were cut into ½-inch lengths, placed in 95-percent alcohol, sealed, and stored until analyzed.

In preparing the roots for analysis the alcohol was drained off and the root material allowed to air-dry, after which it was coarsely ground, returned to the original alcohol, and mixed well. The ground material absorbed the alcohol, thus reincorporating the alcohol-soluble carbohydrates. The alcohol was allowed to evaporate and the dried material was reground until approximately 95 percent would pass through a ½-mm. sieve. This material was used for analyses of carbohydrates and total nitrogen.

LABORATORY DETERMINATIONS

Results for all the analytical data are reported on a dry-weight basis.

The carbohydrate determinations were made from 2-gm. samples of the finely ground root and crown-bud material, and the reducing power of aliquot portions was determined in duplicate. The carbohydrate separates were determined by the cuprous titration method of Shaffer and Hartmann (*23*), and the amounts were calculated as dextrose from the Munson-Walker tables (*1*). Separation was accomplished by heating at approximately 80° C., with 90-percent alcohol, on a sand bath for 1½ hours. One aliquot of the filtrate was used to determine reducing sugars, and a second aliquot was hydrolyzed for three-quarters of an hour with 2½-percent hydrochloric acid for total sugars. The dextrins were separated from the residue with cold 10-percent alcohol, and an aliquot was hydrolyzed with 10 cc. of hydrochloric acid (1.19) for 2½ hours. The residue was washed into a beaker with hot water, boiled for 2 minutes, cooled, digested with saliva at 38° for 14 hours, filtered, and an aliquot portion hydrolyzed with 10 cc. of hydrochloric acid (1.19) for 2½ hours for starch. The residue was then hydrolyzed with 100 cc. of 1+20 hydrochloric acid for hemicelluloses.

Bound water was determined, by the dilatometer method as described by Rosa (*22*), on duplicate 10-gm. samples of the fresh crown-bud material. Petroleum ether was placed in the dilatometer with the samples, care being taken to exclude all air. The cooling was done by placing the filled dilatometers in calorimeters filled with cracked ice and salt.

Specific conductance determinations were made on duplicate 3-gm. samples frozen for 2 hours at −6° to −8° C. and exosmosed 4 hours in 60 cc. of distilled water. The specific conductance was reported in mhos, determined by the use of Student's potentiometer as a conductivity bridge. Dexter et al. (*3*) described this method and used it to measure the degree of cold resistance in plant material.

EXPERIMENTAL RESULTS

CROWN-BUD DEVELOPMENT

The growth and development of the crown buds of alfalfa were traced by taking samples from the experiment outlined in table 1. Samples were collected at intervals of 10 to 30 days from September 1935 to January 1936. The data covering the 1936 season are presented in figure 1 as averages for the dry weight of buds from plots 1 to 5 inclusive. These data show that the buds on the plants during the summer months are very few and small, but that they begin to increase in both number and size in September and continue to do so into November.

Since the buds that develop in the fall produce the next season's hay crop, conditions that favor the formation of numerous strong, healthy buds in the fall may aid in producing a larger hay yield the following season (*7*, *12*).

In the vicinity of Manhattan, Kans., top growth of alfalfa stops increasing in quantity about October 5 to 10. From that time until killing frosts occur, the plant continues to manufacture food and store it in the roots, a peak in storage being reached sometime in November.

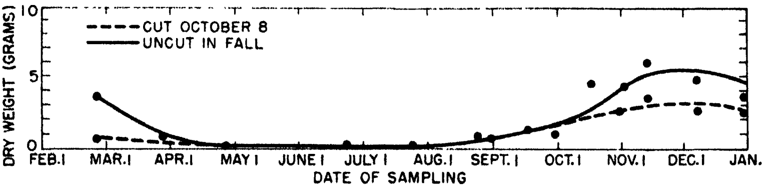

FIGURE 1.—Dry weight of crown buds from 100 alfalfa plants as affected by the fall cutting treatment outlined in table 1.

The actual amounts of crown buds taken from 100 plants on plots 1 to 5 are shown in figure 2. The August 26 samples (fig. 2, *A*) were taken at about the time the buds began to develop, and the November 17 samples (fig. 2, *B* and *C*) were taken after all top growth had been stopped by freezing. The *B* samples were from the plots cut October 8 and the *C* samples from the uncut plots (table 1).

FOOD RESERVES

The fluctuations in the reserves of alfalfa roots throughout the growing season and the effect of the length of the fall growing period on the total amount of food reserves stored for winter use have already been reported (*7*). The cutting practices that were favorable to high plant-food storage were also found to be favorable to crown-bud development.

The present experiment was originally planned to determine the effect of fall cutting treatments on the processes that harden Kansas Common and Ladak alfalfa to cold. Because of drought in the falls of 1937 to 1940, however, the amount of fall growth was so affected that no significant differences due to cutting treatments were found either in the top growth or in the food reserves. The severity of the drought in the fall of 1939 made sampling impracticable. Since the varietal differences between Kansas Common and Ladak were slight,

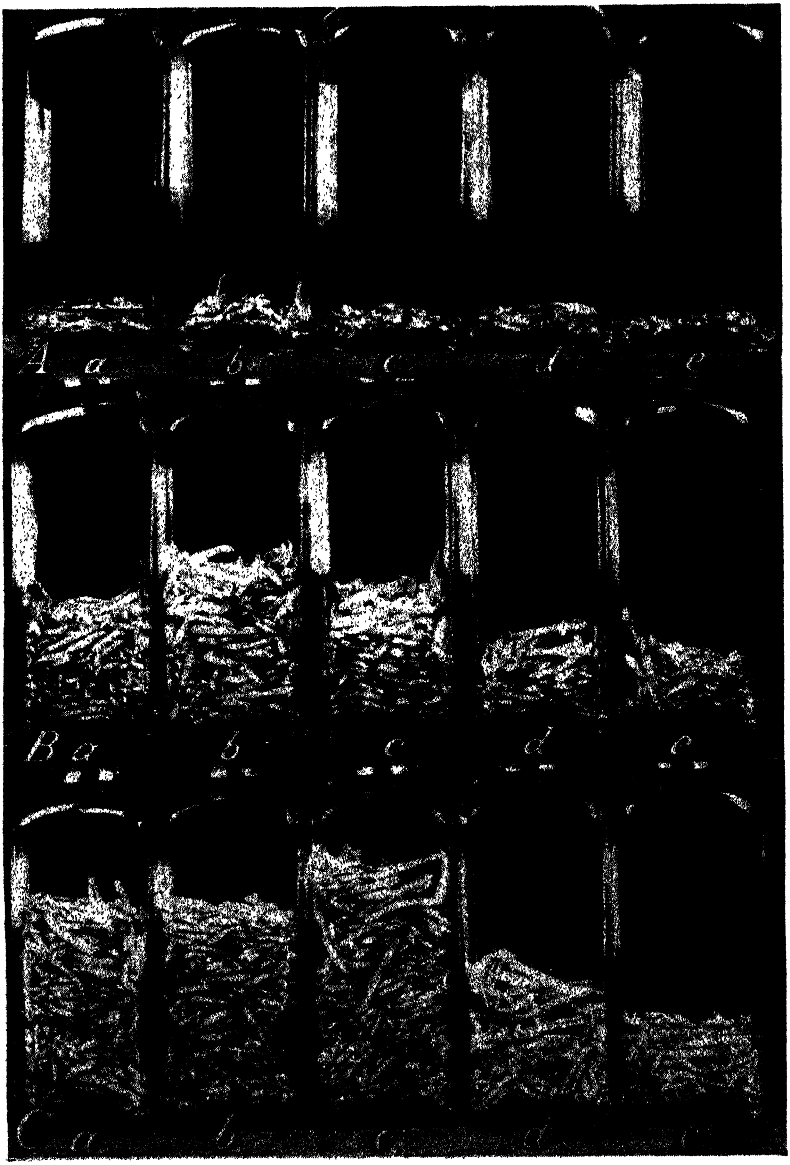

FIGURE 2.—Effect of cutting practices on development of alfalfa crown buds from plots 1 to 5 (*a-e*), as outlined in table 1: *A*, Samples taken at about the time the buds began to develop; *B* and *C*, samples taken November 17, after all top growth had been stopped by freezing, (*B*) from plots cut October 8 and (*C*) from uncut plots.

the data from the carbohydrate analyses for the two varieties were averaged (table 2). However, there was some indication that the hydrolysis of the stored starch to sugars in the roots during the hardening period was more complete in Ladak than in Kansas Common.

The data for total carbohydrates, starch, and total sugars are plotted as the deviation from the mean at the dates sampled (fig. 3). Each dot represents the average of eight field samples taken on approximately the dates indicated in each of the years 1937, 1938, and 1940.

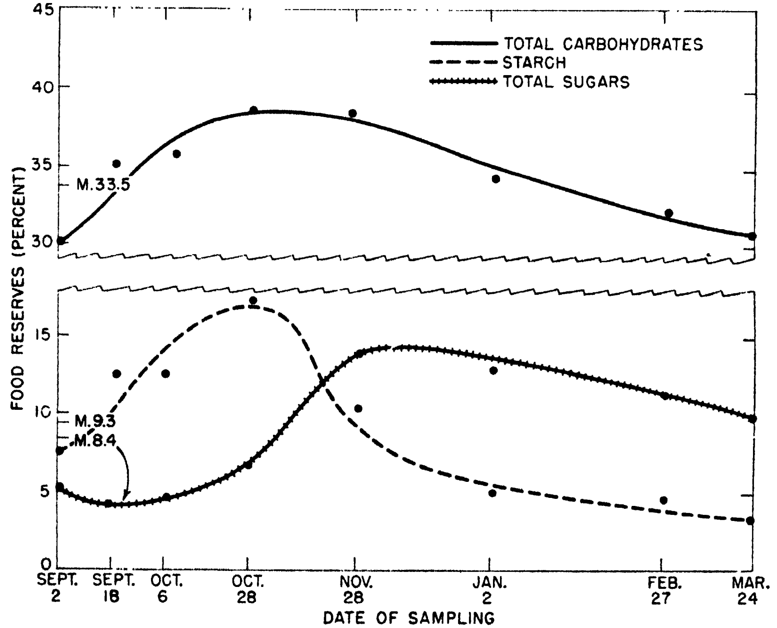

FIGURE 3.—The trend of plant food reserves in alfalfa roots, plotted as deviations from the mean.

Only total carbohydrates, starch, and total sugars are plotted, as the other determinations were more or less constant throughout the season (table 2). In 1937 hemicellulose and dextrin were not separated from the starch. In figuring the deviation for starch that year, it was assumed that all the fluctuations that took place were in the starch. No doubt there is some error in this assumption, but, as indicated by the 1938 and 1940 data, the fluctuation in starch was by far the greater, and by comparison the changes in hemicellulose and dextrins were not large enough to be important.

Most of the carbohydrates found in the crown buds of the alfalfa plant are probably translocated from the roots, as the buds are not storage organs.

The analytical data reported for the crown buds (table 2) were obtained from the same plants that were used for the root analysis. In figure 4 the percentages of total carbohydrates, starch, and total sugars are plotted as the deviation from the mean.

TABLE 2.—*Carbohydrates and nitrogen in roots and crown buds of alfalfa plants sampled at different dates*

PERCENTAGE OF CARBOHYDRATES IN ROOTS

Constituent and year	Date of sampling								Average
	Sept. 2	Sept. 18	Oct. 6	Oct. 28	Nov. 28	Jan. 2	Feb. 27	Mar. 24	
Total carbohydrates:									
1937–38	28.70	26.55	33.75	39.20	39.05	34.10		28.75	32.87
1938–39	29.45	35.15	31.85	37.00	37.85	35.40	32.95	31.50	33.89
1940–41		28.80	37.70		38.55	33.20			34.56
Starch:									
1937–38 [1]	25.80	24.50	30.75	34.50	29.20	20.80		10.05	25.09
1938–39	6.82	16.53	12.46	18.18	8.93	3.94	5.49	5.33	9.71
1940–41		8.18	12.07		7.55	6.88			8.67
Total sugars:									
1937–38	2.92	2.07	3.05	4.80	9.85	13.20		9.70	6.51
1938–39	7.71	4.74	5.59	7.08	14.87	14.76	12.68	9.68	9.64
1940–41		4.71	6.53		12.51	9.43			8.30
Hemicellulose:									
1938–39	14.05	12.60	13.30	11.80	11.90	12.25	13.75	15.60	13.16
1940–41		13.20	13.65		12.65	13.05			13.14
Reducing sugars:									
1937–38	1.75	1.92	2.23	3.86	4.03	8.65		4.47	3.84
1938–39	6.36	3.88	3.27	3.83	4.36	5.73	2.71	3.81	4.24
1940–41		3.49	4.41		4.54	2.92			3.84
Dextrins:									
1938–39	.89	.97	.39	1.27	2.03	1.28	.99	.98	1.10
1940–41		1.16	1.04		.91	.87			1.00

PERCENTAGE OF CARBOHYDRATES IN CROWN BUDS

Constituent and year	Sept. 2	Sept. 18	Oct. 6	Oct. 28	Nov. 28	Jan. 2	Feb. 27	Mar. 24	Average
Total carbohydrates:									
1937–38	20.95	21.80	23.10	25.15	26.10	30.50		25.25	24.69
1938–39	16.65	19.30	19.85	22.65	29.65	32.25	29.95	20.85	23.89
1940–41		15.30	23.00		22.80	23.55			21.16
Starch:									
1937–38 [1]	18.00	18.40	19.80	18.55	17.20	15.65		18.05	17.95
1938–39	2.15	3.43	.72	1.40	.33	.50	1.01	.55	1.26
1940–41		7.34	7.37		2.89	3.57			5.29
Total sugars:									
1937–38	3.41	3.35	3.31	6.59	8.90	14.83		7.33	6.82
1938–39	3.51	3.53	5.59	7.68	17.75	18.12	15.33	6.57	9.76
1940–41		4.77	3.68		8.51	9.07			6.51
Hemicellulose:									
1938–39	10.30	11.45	12.75	12.35	10.70	11.90	11.60	12.85	11.74
1940–41		1.76	10.47		10.59	7.83			7.66
Reducing sugars:									
1937–38	1.46	2.17	2.10	3.19	2.73	7.35		2.77	3.11
1938–39	1.50	1.64	1.58	2.65	2.15	2.05	2.48	1.66	1.96
1940–41		1.44	1.42		.98	1.83			1.42
Dextrins:									
1938–39	.56	1.10	.77	1.15	.87	1.72	1.97	.92	1.13
1940–41		[2] T	.46		.78	.89			.71

PERCENTAGE OF NITROGEN IN ROOTS

Constituent and year	Sept. 2	Sept. 18	Oct. 6	Oct. 28	Nov. 28	Jan. 2	Feb. 27	Mar. 24	Average
Total nitrogen:									
1937–38	1.90	1.92	2.45	2.06	2.21	2.13		2.24	2.13
1938–39	1.38	1.87	1.70	1.92	2.06	2.12	2.29	2.30	1.96
1940–41		1.54	1.65		2.01	2.22			1.86

PERCENTAGE OF NITROGEN IN CROWN BUDS

Constituent and year	Sept. 2	Sept. 18	Oct. 6	Oct. 28	Nov. 28	Jan. 2	Feb. 27	Mar. 24	Average
Total nitrogen:									
1937–38	3.60	4.13	4.39	4.89	4.13	3.78		4.14	4.08
1938–39	3.85	4.45	4.14	4.56	4.30	3.69	4.09	4.04	4.14
1940–41		4.65	4.43		4.12	4.18			4.35

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[1] During this season hemicellulose and dextrins were not separated from starch.

[2] T = trace.

The percentage data for total nitrogen are likewise given in table 2. From September to March, the general trend in the roots was upward, with an average of 1.98 percent. In the buds the average was 4.19 percent, the highest percentages being reached from late September through November.

THE HARDENING PROCESS

Changes in Carbohydrates

Changes in total carbohydrates are due largely to changes in the starch and total sugar content. In the roots, a large proportion of the total carbohydrates is stored as starch, whereas in the crown buds total sugars account for the largest proportion, and starch is present only in limited amounts (figs. 3 and 4). It is shown that storage in the roots continued until the latter part of October, when starch reached its maximum concentration. After that time starch decreased and total sugars increased, reaching a maximum in the roots during December. The maximum concentration of total sugars in the crown buds occurred during December and January and coincided with a rapid decrease of starch in the roots. Since little starch was present in the crown buds during the late fall and winter, the increase in sugar concentration was probably due to translocation from the root or crown tissue to the buds. Hydrolysis of starch and translocation of sugar occurred during the period when the plant was increasing in cold hardiness, and appeared to be a factor associated with the hardening process.

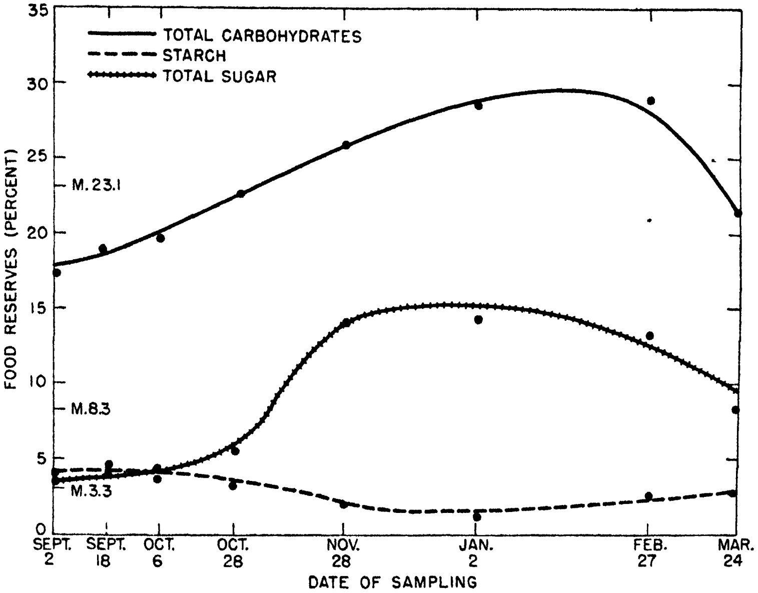

Figure 4.—Trend of plant-food reserves in alfalfa crown buds, plotted as deviations from the mean.

CHANGES IN NITROGEN

The total nitrogen content of the crown buds was found to average more than 2 percent higher than that of the roots, thus assuring sufficient nitrogen for the formation of proteins, which are largely colloidal in nature.

OTHER PHYSIOLOGICAL CHANGES

Changes in dry weight, free water, bound water, and specific conductance of various plant parts of crop plants, including alfalfa roots, have been reported by a number of investigators. So far as the writer has been able to determine, however, no one has reported work on the crown buds of alfalfa and their relation to the storage organs in the utilization of stored carbohydrate and nitrogen reserves during the process of hardening to cold. Data here presented (table 3; fig. 5) indicate an increase in dry weight of the buds from September to January, after which the dry weight gradually decreased. Total water increased through the early fall, began to decrease the latter part of October, and reached a minimum in January, at a time when the buds exhibit greatest resistance to cold, as indicated by minimum values in specific conductance. The trend of the curve for bound water, calculated as percentage of total water, is opposite to that of total water, and there are no reverse changes in the early fall, as in the case of total water. The rapid increase in the percentage of bound water from October 6 to January 2 coincides with an increase in

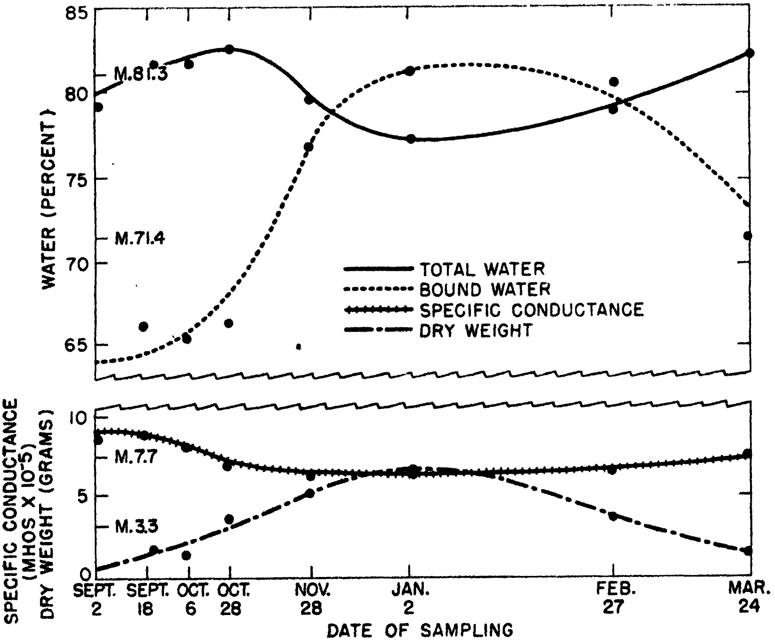

FIGURE 5.—Physiological changes in alfalfa crown buds, plotted as deviations from the mean.

cold resistance and with an increase in sugar. From table 3 it may be shown that approximately 20 percent of the total water from November to March remained as free water.

TABLE 3.—*Some physiological changes in crown buds of alfalfa plants at different dates of sampling*

Item and year	Dry weight, amount of water, and specific conductance in samples taken on indicated date								
	Sept. 2	Sept. 18	Oct. 6	Oct. 28	Nov. 28	Jan. 2	Feb. 27	Mar. 24	Average
Dry weight:	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
1937-38	0.82	1.62	2.73	3.24	2.94	3.62	---	1.79	2.30
1938-39	.93	2.74	2.28	4.45	7.09	9.86	4.86	2.06	4.28
Total water:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1937-38	76.9	81.3	81.4	83.7	80.0	76.4	---	81.21	80.1
1938-39	80.1	79.3	79.6	79.8	77.8	74.9	78.4	83.4	79.2
1940-41		84.9	84.7	---	82.2	81.5	---	---	83.3
Bound water:[1]									
1937-38	74.0	86.8	74.1	72.1	85.1	90.7	---	80.1	80.4
1938-39	64.5	63.2	62.3	72.9	83.6	89.4	84.1	75.2	74.4
1940-41	---	49.1	60.3	---	62.9	65.8	---	---	59.5
	Mhos ($\times 10^{-5}$)	*Mhos* ($\times 10^{-5}$)	*Mhos* ($\times 10^{-5}$)	*Mhos* ($\times 10^{-5}$)	*Mhos* ($\times 10^{-5}$)	*Mhos* ($\times 10^{-5}$)	*Mhos* ($\times 10^{-5}$)	*Mhos* ($\times 10^{-5}$)	*Mhos* ($\times 10^{-5}$)
Specific conductance:									
1937-38	7.2	10.0	8.0	7.8	6.3	5.7		8.6	7.7
1938-39	9.6	8.7	7.5	6.5	6.2	5.5	5.9	6.1	7.0
1940-41	---	7.8	9.5	---	7.1	9.2		---	8.4

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[1] Calculated as percentage of total water.

Specific conductance has been used as an indicator of cold resistance in crop plants. Data shown in table 3 and figure 6 indicate the trend in the crown buds toward increasing hardiness, and correlate with the sugar content, total water, and bound water. All analytical data show more fluctuation between sampling dates in the fall than at any other time. This fluctuation may be caused by the rapid changes that often occur in growing conditions, such as alternate wet and dry or warm and cold periods during this season. When alfalfa is growing rapidly the carbohydrates in the roots decrease, whereas under conditions that limit vegetative growth, such as low soil moisture or low temperatures, photosynthesis continues, resulting in a rapid increase of reserves.

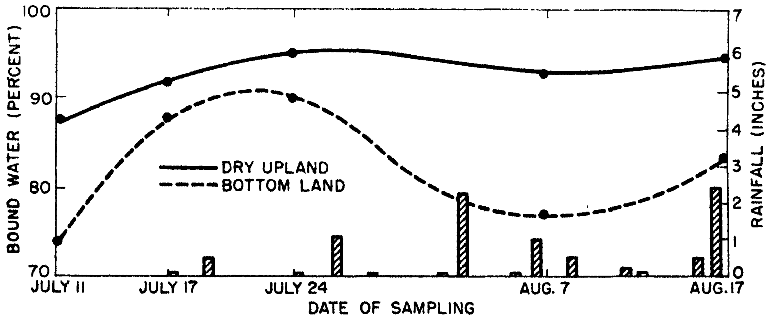

FIGURE 6.—Effect of moisture conditions on percentage of bound water in alfalfa roots.

This fluctuation is reflected in all the data, indicating the influence of stored food on the other factors.

Environmental factors that tend to increase the dormancy of the alfalfa plant, regardless of the time in the growing season, will tend to increase its resistance to unfavorable conditions, bringing about physiological changes that makes it more resistant to cold in the winter (*2*, *7*, *13*) and to drought (*14*, *18*, *26*) in the summer. During the summer of 1939 data were obtained on the effect of moisture conditions on the bound water in alfalfa roots, and these are presented in figure 6. Observations had shown that there was a difference in the effect of available moisture on the growth of alfalfa tops in the two adjoining areas sampled. In June before the first sampling date, growing conditions were good, the rainfall of 7.3 inches for the month being fairly evenly distributed. Between July 2 and 17 (fig. 6) no rain fell, and the mean maximum temperature was 100° F. for the 15 days, causing the bound water to increase rapidly. After July 17, there were again light rains and until August 7 growing conditions were favorable on the bottom land because of the combined effect of the rainfall and the runoff from the upland. During this period the bound water decreased. Because of the rapid runoff, the plants on the upland received very little benefit from the rainfall. Between August 7 and 16 the weather was again hot and dry, and this condition was soon reflected in the bound-water content of the roots from the bottom land. The weather changes were not sufficient to exert much effect on the bound water in the roots of the upland samples.

DISCUSSION

Earlier reports on work in this series of experiments (*6*, *7*) have shown that the carbohydrate content and the nitrogen content of the roots rise and fall with the development of new growth in the spring or after cutting. It has also been shown (*7*) that when top-growth conditions in the fall are favorable for high food reserves and for the development of numerous and vigorous crown buds, there is a more vigorous top growth the following spring and an increase in the yield of hay from the first cutting.

The physiological factors recorded in figure 5 suggest that the increase of carbohydrates in roots and buds and the high nitrogen content of the buds (table 2) are closely associated with the increase in bound water and the reduction in free water. It is definitely known that proteins are not formed in the plant without a supply of carbohydrates as a constituent, as well as a form of energy necessary for the reduction of nitrogen (*16*). During the hardening period, between October 6 and November 28, the rapid translocation of sugars from the roots increased the concentration of the cell sap in the buds, which tended to lower the percentage of total water. From October 6 to January 2 there was also a 20-percent increase in the amount of total water that became bound water. An additional factor in the reduction of free water was probably the high carbohydrate content in both the roots and the buds, which supplied the necessary energy for the reduction of the nitrogen, thus increasing the colloidal content of the buds.

SUMMARY

The time of fall cutting has been shown to have a direct influence on the physiological processes that take place in the alfalfa plant.

Fall cutting experiments conducted between 1935 and 1940 dealt directly with the processes of the alfalfa plant that influence the initiation and growth of the crown buds and their relation to cold resistance. The factors studied included food reserves, bound water, specific conductance of exosmosed material, total water, and dry matter.

Both the number and the development of the crown buds were influenced by the amount of fall top growth remaining on the plants during the fall, indicating the importance of making the last cutting early enough to allow at least 6 inches of top growth to develop before the beginning of the fall dormant period.

Cutting practices favorable to high food storage were also favorable to crown-bud development. Cutting practices are also closely correlated with the hardening processes, as shown by the amount of total water, bound water, and specific conductance.

The hardening period, as shown by these determinations, extends from approximately September 1 to December 1, the most rapid changes taking place between October 1 and December 1. Some changes indicate that increased resistance occurred as late as January 1. During the hardening period a rapid hydrolysis of starch, the principal form of plant food storage, occurred, with a subsequent translocation of the resulting sugar to the crown buds.

The average amount of total nitrogen in the roots was 1.98 percent, dry weight, and in the crown buds, 4.19 percent. The high nitrogen content of the crown buds may account in part for their high content of bound water.

The decrease in percentage of total water was associated with the increased concentration of sugars and the high nitrogen content of the buds, which resulted in the further binding of the free water with protoplasmic compounds.

The decrease in the amount of free water to approximately 20 percent of the total water and the increased concentration of sugars lowered the freezing point of the crown-bud tissue, as shown by the specific conductance test.

The data presented in these and earlier experiments indicate that food reserves are essential for developing cold resistance in alfalfa and from a practical standpoint can be influenced by proper cutting practices.

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MUSTARD OILS IN CRUCIFERS AND THEIR RELATION TO RESISTANCE TO CLUBROOT[1]

By MARK A. STAHMANN, *research associate*,[2] KARL PAUL LINK, *professor, Department of Biochemistry*, and J. C. WALKER, *professor*,[2] *Department of Plant Pathology, Wisconsin Agricultural Experiment Station*

INTRODUCTION

It has been shown by a number of workers (*7, 23, 30, 31*)[3] that there exists within the Cruciferae a wide range of resistance and susceptibility to the clubroot organism (*Plasmodiophora brassicae* Wor.). After a rather extensive anatomical study of the root structure of resistant and susceptible varieties, Rochlin (*23, 24*) showed that there is no basis for resistance to be found in the root morphology. She decided, because of an apparent correlation between mustard-oil content and resistance, that the mustard oil in certain varieties and species was responsible for their resistance to clubroot on the basis of the high toxicity of the isothiocyanates to micro-organisms (*14, 6, 22, 33*). The correlation was indicated in a table listing a number of species and varieties of crucifers, the percentage of infected plants recorded for each by various workers, and the relative amount of mustard oils reported for the same forms. It should be pointed out that in many cases where mustard oils were listed as absent, a search of the literature has revealed reports of the identification of mustard oils in these species. Rochlin listed the absence of mustard oils in each of 3 varieties of cabbage (*Brassica oleracea* var. *capitata* L.) which she investigated. On the other hand, Grimme (*10*) reported the presence of mustard oils in each of 10 varieties of cabbage which he studied. Likewise, Rochlin listed the absence of mustard oils in *Sinapis arvensis*, but Rothea (*25*) reported mustard oils in this slant. The evidence which appeared to be the real basis for Rochlin's explanation consisted principally of an experiment in which the volatile mustard-oil content of a number of resistant and susceptible varieities was roughly estimated. This was done by noting the relative strength of the pungent odor arising from root-tissue macerates of resistant and susceptible plants. A correlation between the relative strength of the mustard-oil odor and resistance was reported.

In view of the conclusions reached by Rochlin, it was decided to conduct a biochemical study of the mustard-oil fraction from the root tissues of varieties and species resistant and susceptible to clubroot to

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[3] Italic numbers in parentheses refer to Literature Cited, p. 62.

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determine whether the isothiocyanates were a factor in determining this resistance. The present paper is one of a series having to do with resistance to clubroot in certain forms of black mustard (*Brassica nigra* (L.) Koch) and turnip (*B. rapa* L.). The determination of the relative resistance to clubroot of these forms has been reported elsewhere (*30, 31*). Other species included for comparative study were white mustard (*B. alba* (L.) Boiss.), cabbage (*B. oleracea* var. *capitata* L.), and horseradish (*Armoracia rusticana* Gaertn.).

The first phase of this study represents a qualitative investigation of the mustard oils of resistant and susceptible forms to ascertain whether resistance could be ascribed to the presence of specific mustard oils. The second phase deals with the development of a chemical method of analysis for the estimation of mustard oils in plant tissue, and the application of this method to the quantitative determination of the mustard-oil content of resistant and susceptible root tissues. The third part is a study of the mustard-oil enzyme system in resistant and susceptible plants.

EXPERIMENTAL RESULTS

ISOLATION OF MUSTARD OILS

The general procedure used was first worked out on the cortical[4] tissue of mature fleshy roots of turnip. This scheme with some modifications was later applied to the root tissues of other crucifers.

Three kilograms of the fresh tissue, peeled from the roots, was ground to a pulp in a Nixtamal mill. The pulp was divided into four 750-gm. portions and each portion placed in a 5-liter steam distillation flask containing 1,500 ml. of water. A rapid exhaustive steam distillation was immediately carried out. Since the hydrolysis of the mustard-oil glycosides in root tissues occurs rather rapidly, distillation immediately after grinding usually gave slightly higher yields of mustard oil than distillation after a 3-hour period of maceration. After a 12-hour maceration period, yields were reduced to about one-fourth of the amount that could be obtained immediately after grinding.

Since the amount of plant tissue available for this study was limited, it was decided to purify the mustard-oil fraction by crystallization of the corresponding substituted thiourea rather than by fractional distillation of the very small amount of mustard oil that would be obtained from the steam distillation. Accordingly, the distillate was collected in 2-liter receivers containing 25 ml. of concentrated ammonium hydroxide. To prevent loss of the volatile mustard oils, the condenser was fitted with an adapter which dipped below the level of the liquid in the receiver. From 3 kg. of tissue, about 6–liters of distillate was collected. The distillate containing excess ammonium hydroxide was allowed to stand at room temperature for 12 hours to complete thiourea formation, and then extracted 12 hours with peroxide-free ethyl ether in a continuous liquid-liquid extractor.

The ether solution from 6 kg. of the cortical tissue of turnip roots was concentrated to about 100 ml., 200 ml. of 95-percent ethanol added, and the remaining ether removed by distillation. The light yellow alcoholic solution containing the thiourea fraction corresponding to the mustard oils in the distillate was treated with a small amount of carbon to remove some of the color. An excess of carbon was avoided since it might adsorb significant amounts of the thiourea fraction. The alcoholic filtrate was concentrated under reduced pressure to about 25 ml. and transferred to a crystallizing dish. The solution was then heated on a steam bath and hot water added to the first point of turbidity. On cooling and slowly removing the alcohol by concentrating in a vacuum desiccator, crystalline, diamond-shaped plates separated. This crude, water-insoluble thiourea fraction was collected by filtration in yields from 0.2 to 0.8 gm. depending on the variety used. This product was recrystallized from ethanol-water mixtures to a constant melting point, 136° to 137° C. Repeated attempts were made to isolate other thioureas from the mother liquors by extraction with different solvents, fractional crystallization, and high vacuum distillation, but only the one product could be obtained.

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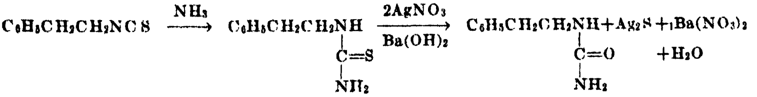
[4] The term "cortical" as used in this paper refers to the tissue of the turnip fleshy root obtained when an outer peel about 2 mm. in thickness was removed. Anatomically such tissue is not primary cortex but periderm, phloem, and possibly some secondary xylem

CHARACTERIZATION OF MUSTARD OILS

The melting point could not distinguish between the thiourea obtained from *d*-secondary-butyl isothiocyanate, melting point 134° C., and that from β-phenethyl isothiocyanate, melting point 137° C. Both mustard oils have been isolated from crucifers (*27*). The mixed melting point with a synthetic sample of β-phenethyl thiourea, the elementary analysis, and conversion of the isolated thiourea to the corresponding urea were used to distinguish between these two possible thioureas. Accordingly, a portion of the thiourea isolated from each species was converted to the substituted urea. In this way two derivatives of mustard oil could be obtained from the same sample.

The conversion of the thiourea to the substituted urea was carried out by a modification of the method of Bertram and Walbaum (*3*). Twenty milligrams of the thiourea was dissolved in 10 ml. of ethanol and a slight excess of 0.1 N barium hydroxide added. A slight excess of an aqueous solution of silver nitrate was then added and the mixture heated on a steam bath for 1 hour. The silver sulfide was removed by filtration and the urea extracted from the aqueous alcohol filtrate by ethyl ether. The ether solution was concentrated to about 2 ml. and 10 ml. of hot 95-percent ethanol was added. As the solution cooled and slowly concentrated in a vacuum desiccator, long needles separated. These were collected in a centrifuge tube, washed with an ethanol-water mixture, and dried. The melting point was 112° C., which is the reported melting point of β-phenethylurea (*3*). The reactions involved in the isolation and characterization of β-phenethyl isothiocyanate follow:



$$C_6H_5CH_2CH_2NCS \xrightarrow{NH_3} \begin{array}{c} C_6H_5CH_2CH_2NH \\ | \\ C{=}S \\ | \\ NH_2 \end{array} \xrightarrow[Ba(OH)_2]{2AgNO_3} \begin{array}{c} C_6H_5CH_2CH_2NH \\ | \\ C{=}O \\ | \\ NH_2 \end{array} + Ag_2S + Ba(NO_3)_2 + H_2O$$

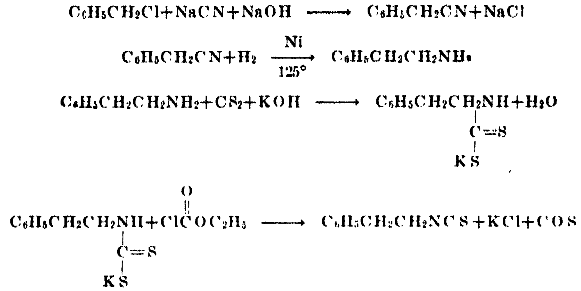
β-phenethyl isothiocyanate β-phenethylthiourea β-phenethylurea

Analysis of β-phenethylthiourea (isolated) $C_9H_{12}N_2S$

Calculated:	C–60.00	H–6.66	N–15.55	S–17.77
Found:	C–60.54, 60.57	H–6.58, 6.70	N–15.85, 15.74	S–17.85, 17.72

SYNTHESIS OF MUSTARD OILS

For purposes of comparison with the material isolated from root tissues and for a study of its relative toxicity to fungi, a sample of β-phenethyl isothiocyanate was synthesized according to the following scheme:



$$C_6H_5CH_2Cl+NaCN+NaOH \longrightarrow C_6H_5CH_2CN+NaCl$$

$$C_6H_5CH_2CN+H_2 \xrightarrow[125^\circ]{Ni} C_6H_5CH_2CH_2NH_2$$

$$C_6H_5CH_2CH_2NH_2+CS_2+KOH \longrightarrow \begin{array}{l}C_6H_5CH_2CH_2NH+H_2O\\ \qquad\qquad\quad |\\ \qquad\qquad\quad C{=}S\\ \qquad\qquad\quad |\\ \qquad\qquad\quad KS\end{array}$$

$$\begin{array}{l}C_6H_5CH_2CH_2NH\\ \qquad\qquad\quad |\\ \qquad\qquad\quad C{=}S\\ \qquad\qquad\quad |\\ \qquad\qquad\quad KS\end{array}+Cl\overset{O}{\overset{\|}{C}}OC_2H_5 \longrightarrow C_6H_5CH_2CH_2NCS+KCl+COS$$

Benzyl cyanide was prepared according to the method of Adams and Thal (*9*). This was reduced to β-phenethylamine as described by Adkins (*1*). β-phenethyl isothiocyanate was prepared from β-phenethylamine according to the procedure of Slotta et al. (*28*).

To a solution of 9 gm. of potassium hydroxide in 50 ml. of water under a reflux condenser was added 20 gm. of β-phenethylamine and then 12 gm. of carbon disulfide. The flask was cooled in a water bath. After the reaction was complete (usually 10 to 15 minutes), 16 gm. of ethyl chloroformate was slowly added. The flask was shaken occasionally to mix the contents and heated under reflux for 30 minutes. The crude mustard-oil layer was then removed in a separatory funnel, dried over calcium chloride, and distilled under reduced pressure. The fraction boiling at 127° to 130° C. at 6 mm. was collected. From 88 gm. of β-phenethylamine, 50 gm. of β-phenethyl isothiocyanate was obtained, a 26-percent yield.

The thiourea was prepared from the mustard oil by adding an excess of concentrated ammonium hydroxide to an alcoholic solution of the mustard oil. Five gm. of β-phenethyl isothiocyanate was dissolved in 20 ml. of 95-percent ethanol and 50 ml. of concentrated ammonium hydroxide added. The mixture was refluxed for 1 hour, concentrated to 25 ml. under reduced pressure, and the crystalline product collected. The yield was 5.4 gm. or 98 percent. After recrystallization from ethanol water the melting point was 136° to 137° C. Since a mixed melting point with the thiourea isolated from turnip tissue showed no depression, the isolated product must have been identical with the synthetic product.

Analysis of β-phenethylthiourea (*synthetic*) $C_9H_{12}N_2S$

Calculated:	C–60.00	H–6.66	N–15.55	S–17.77
Found:	C–60.03, 60.54	H–6.60, 6.65	N–15.56, 15.64	S–17.77, 17.81

QUALITATIVE RELATIONSHIP OF MUSTARD OILS TO RESISTANCE

Since it has been claimed that resistance to clubroot could be correlated with the presence of a *characteristic* volatile mustard oil in the root tissue of resistant crucifers (*23*, *24*), it was thought desirable to identify the mustard oils existing in the roots of representative varieties of different species, resistant and susceptible to clubroot.

It should be pointed out that the isolated yield of crystalline thiourea is not to be considered an exact representation of the quantity of mustard oil or mustard-oil glycoside present in the plant tissue. Loss of mustard oil may occur through hydrolysis or by reaction of the isothiocyanate group with other compounds in the tissue macerate during the distillation. The amount of other volatile oils accompanying the mustard-oil fraction fluctuated with the plant being studied. This variation made differing amounts of purification necessary for the isolation of crystalline thiourea. In some cases one or two recrystallizations yielded the pure thiourea. In other cases repeated recrystallization and decolorization with carbon or a high-vacuum distillation were necessary before the thiourea could be separated from traces of an accompanying yellow oil. Hence, losses during isolation are not proportionate or always comparable. Additions to turnip-tissue macerates of β-phenethyl isothiocyanate in amounts from one-half to three times that previously isolated indicated that about 50 percent of that added could be recovered as crystalline thiourea by this isolation method.

The mustard-oil fraction from the root tissue of the other crucifers was isolated as the substituted thiourea by the method described for turnip tissue, except that from 6 to 15 kg. of the whole, fresh roots were ground and subjected to the steam distillation. In all the varieties investigated in this study except horseradish, the mustard oil isolated was characterized as β-phenethyl isothiocyanate by (1) the melting point of the crystalline thiourea, 136° to 137° C., (2) the melting point of a mixture of the isolated thiourea with an authentic sample of β-phenethylthiourea, and (3) the melting point, 112° C., of the urea obtained from the isolated thiourea in the manner described for the material from turnip tissue.

With all forms studied repeated attempts were made to separate other mustard oils as their thioureas. But in only one case (horseradish) could a second mustard oil be obtained from root tissue. The small amount of total solids in the mother liquors and their low sulfur content would indicate that, if other mustard oils were present, they were to be found only in comparatively minute quantities. That these isolation methods were capable of detecting other mustard oils was shown by the recovery from horseradish tissue of a second thiourea, that corresponding to allyl isothiocyanate. When allyl isothiocyanate was added to turnip-tissue macerates in amounts approximately equal to the β-phenethyl isothiocyanate content of the tissue, approximately half of the added isothiocyanate could be recovered as allyl thiourea after removing the β-phenethylthiourea.

The species and varieties examined in this study and the amounts of mustard oil recovered as thiourea are given in table 1. β-phenethyl isothiocyanate was isolated and characterized from fresh and from dried turnip root tissue. This mustard oil was isolated from each of four strains of black mustard and from one strain each of cabbage, white mustard, and horseradish. Thus the same mustard oil has been shown to be the principal one present in the root tissues of both resistant and susceptible varieties of black mustard and turnip; in

TABLE 1.—*The mustard oils isolated from roots of various crucifers differing in their resistance to clubroot*

Species	Variety or collection	Resistant or susceptible to clubroot	Amount of thiourea obtained per kilogram of fresh tissue	
			β-phenethyl	Allyl
			Milligrams	*Milligrams*
Turnip (*Brassica rapa*)	Purple Top Milan	Resistant	245	0
	Shogoin	Susceptible	85	0
Black mustard (*Brassica nigra*)	3	Resistant	33	0
	10	do	30	0
	8	Susceptible	28	0
	14	do	39	0
White mustard (*Brassica alba*)	15	do	54	0
Cabbage (*Brassica oleracea capitata*).	Jersey Queen	do	104	0
Horseradish (*Armoracia rusticana*)		Resistant	137	100

cabbage and white mustard root tissue, where all varieties known are susceptible; in the root tissue of horseradish, which is usually reported as highly resistant; and in radish root tissue. It is evident, therefore, that this mustard oil is characteristic of root tissues of many crucifers.

In the case of horseradish, whole, mature roots were ground in a hammer mill and then covered with water for 1 hour before distillation. The isolation of β-phenethylthiourea from the distillate was carried out as described for turnip tissue. In addition, allylthiourea was isolated from the aqueous mother liquors after removal of the β-phenthylthiourea. These mother liquors were concentrated to dryness, the residue was dissolved in 100 ml. of hot absolute ethanol, and the solution was decolorized with carbon. On cooling and slowly concentrating in a vacuum desiccator, crystals separated. After recrystallization their melting point was 74° C. This coincides with the melting point of allylthiourea, and a mixed melting point with an authentic sample of allythiourea showed no depression.

The isolation of β-phenethyl isothiocyanate from one variety of turnip root tissue has been previously reported by Kuntze (*15*). The isolation of this mustard oil and allyl iosthiocyanate from horseradish root tissue has been reported by Heiduschka and Zwergal (*12*) and by Pietschmann (*20*). Bertram and Walbaum (*3*) found the former in root tissue mignonette (*Reseda odorata* L.).

QUANTITATIVE ESTIMATION OF MUSTARD OILS IN PLANT TISSUE

Studies of the quantitative methods for the determination of mustard oils were undertaken to develop analytical methods that could be applied to (1) the determination of the total mustard-oil content of root-tissue macerates as an estimation of the potential mustard-oil content of the tissue, and (2) a study of the rate of hydrolysis of a mustard-oil glycoside substrate by tissue macerates as an estimation of the mustard-oil enzyme activity of the tissue. It was thought that if mustard oils were concerned with resistance to clubroot, either the rate of their release by enzymatic hydrolysis when infection began or the total amount available might become limiting factors.

The literature gives various methods for the determination of mustard oils. Most of these have been devised to estimate allyl isothiocyanate in mustard seed or the mustard oil of commerce. The estimation of the small amounts of mustard oil present in root-tissue macerates presented some difficulties that prevented the direct application of any single described method to this problem. In the development of the analytical scheme used in this study, a number of the methods of mustard-oil analysis that have been described in the literature were studied (*2, 4, 8, 11, 16, 17, 20, 29*), purified samples of β-phenethyl and allyl isothiocyanates being used. The final scheme made use of some of the steps in these methods, together with some new techniques. The individual experiments on which each of the steps in these analytical schemes was based will not be described in detail. It will suffice to state that each operation was first studied separately to insure that it could be carried out quantitatively. Then the individual operations were combined and the entire scheme checked against purified mustard oils and a mustard-oil glycoside.

The method finally adopted for the estimation of the total mustard-oil content of root tissue is based on the following steps: (1) Removal of the mustard oil from the tissue macerate by steam distillation, (2) conversion to the thiourea, (3) extraction of the thiourea with ether, and (4) estimation of the thiourea by measuring the silver consumed in precipitating the sulfur by the Volhard titration. It is recognized that this procedure may not give an absolute measure of the mustard-oil glycoside content of the tissue because the enzymatic hydrolysis does not go to completion. Furthermore, losses of mustard oil may occur before or during the distillation by hydrolysis of the isothiocyanates, by condensation with other plant constituents, or by the action of a mustard oil enzyme system. However, it is believed that although the results may not be absolute, they will serve in comparing the total mustard oil or mustard-oil glycoside content of resistant and susceptible crucifers.

Mature turnips were harvested and the fleshy roots washed and peeled. The cortical tissue was sliced and mixed. A sample was taken for the moisture determination. The sliced tissue was then ground to a pulp in a Nixtamal mill. Four 750-gm. portions (3 kg.) were weighed into 5-liter steam-distillation flasks and subjected immediately to exhaustive steam distillation. The condensers were fitted with an adapter and outlet which reached below the level of the liquid in the receiver. The receivers contained 50 ml. of concentrated ammonium hydroxide in 200 ml. of water. Six liters of distillate was collected. The distillate appeared clear during the latter half of the distillation. The distillates were combined, stirred for 2 hours, and allowed to stand 12 hours to complete conversion of the mustard oil to the corresponding thiourea. The thiourea was then extracted from this aqueous solution with peroxide-free ethyl ether in a continuous liquid-liquid extractor. The ether solution was concentrated to about 50 ml. and transferred to a 500-ml. volumetric flask, using 25 ml. of ethanol and about 300 ml. of water to wash the contents of the boiling flask into the volumetric flask. The flask was then heated to about 60° C. to remove the remaining traces of ether. Standard 0.1 N silver nitrate solution (100 ml.) was then added and the contents mixed by swirling the flask. After the reaction mixture had stood 24 hours to complete the sulfide precipitation, the contents of the flask were brought to volume with water, mixed, and the precipitated silver sulfide filtered off through a dry filter paper.

One hundred-milliliter aliquots of the filtrate were acidified with 10 ml. of 3 N nitric acid and titrated with standard 0.1 N ammonium thiocyanate solution ferric-ammonium alum being used as an indicator. The mustard-oil content of the tissue was calculated as β-phenethyl isothiocyanate from the amount of silver removed as silver sulfide.

Mustard roots were analyzed in a similar manner. The fresh, mature roots were dug, washed, and the whole roots ground in a Wiley mill. Because of the limited amount of mustard-root tissue available, the sample size was limited to about 1 kg. Distillation and mustard-oil estimation were carried out as described for turnip tissue.

MUSTARD-OIL CONTENT IN RELATION TO DISEASE RESISTANCE

The results secured with resistant and susceptible turnip and black mustard are given in table 2. It will be seen that no correlation was found between the resistance to clubroot of the strains from which the tissue samples were collected and the chemical estimation of mustard oil.

TABLE 2.—*Mustard-oil content of root tissue of resistant and susceptible turnip and black mustard plants*

Species	Variety or collection	Resistance	Mustard oil [1]
			Percent
Turnip	Snowball	Highly resistant	0.019
	White Milan	do	.015
	Purple Top White Globe	do	.021
	Purple Top Milan	do	.024
	Cowhorn	Moderately resistant	.020
	Shogoin	Very susceptible	.017
Black mustard	3	Resistant	.010
	14	Susceptible	.016

[1] Expressed as β-phenethyl isothiocyanate calculated on fresh weight.

In studies parallel with these, Pryor (*21*) had shown that when resistant mustard and turnip were grown in sand-nutrient cultures in which sulfur was omitted, the presence of mustard oils in the leaves could not be detected by taste, but resistance to clubroot was nevertheless not broken down. It was of interest, therefore, to study the mustard-oil content of resistant and susceptible plants grown with and without sulfur in the nutrient. Stems and leaves were frozen with solid carbon dioxide, ground while frozen, and heated to 37° C. in water for one-half hour before distillation. The distillate was collected in an excess of ammonium hydroxide. After conversion to the thiourea was complete, the distillate was extracted with ether and the analyses carried out in a manner similar to that used with root tissue except that smaller samples and less silver nitrate were used.

The results are given in table 3. It will be seen that in all cases the mustard-oil content was reduced to a trace or a very small amount as compared with that in plants grown in a standard nutrient. Nevertheless, the resistant plants remained resistant, and the susceptible strains were heavily infected, according to Pryor (*21*).

It is clearly evident that the total mustard-oil content of the crucifers studied was not related to their resistance or susceptibility to clubroot. Furthermore, when the oil content was raised or lowered through nutrition, no measurable effect on infection and resistance was to be noted.

TABLE 3.—*Mustard-oil content of the stems and leaves of plants grown in sand-nutrient culture with and without sulfur*

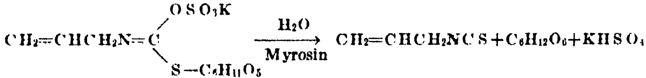
Species	Variety or collection	Resistance	Mustard oil [1] determined in plants grown—	
			With sulfur	Without sulfur
			Percent	*Percent*
Black mustard	10	Resistant	0.026	0.005
	14	Susceptible	.043	.002
Turnip	Purple Top Milan	Resistant	.003	(2)
	Shogoin	Susceptible	.002	(2)
Cabbage	Jersey Queen	do	.004	.001

[1] Expressed as β-phenethyl isothiocyanate calculated on fresh weight.
[2] Trace.

MYROSIN ACTIVITY OF ROOT TISSUE

THE MUSTARD-OIL ENZYME SYSTEM

Mustard oils occur in the plant cells in a conjugated form as glycosides. The best known example of a mustard-oil glycoside is sinigrin (potassium myronate), which on enzymatic hydrolysis yields 1 mole of allyl isothiocyanate, *d*(+)-glucose, and potassium acid sulfate according to the equation:



$$CH_2{=}CHCH_2N{=}C\begin{matrix}\diagup OSO_3K \\ \diagdown S{-}C_6H_{11}O_5\end{matrix} \xrightarrow[\text{Myrosin}]{H_2O} CH_2{=}CHCH_2NCS + C_6H_{12}O_6 + KHSO_4$$

Sinigrin Allyl isothiocyanate Glucose Potassium acid sulfate

The enzyme system that effects this hydrolysis is called myrosin. It has been demonstrated to be widely distributed in the tissues of the crucifers and in some members of other plant families (*27*). Neuberg (*18*), and Neuberg and Wagner (*19*) have shown myrosin to consist of two enzymes, a sulfatase which splits off potassium acid sulfate, and a thioglucosidase which splits the thioglycoside linkage. Sandberg and Holly (*26*) have shown by glucose determinations that hydrolysis by thioglucosidase proceeds only to a definite equilibrium value (66 percent), but sulfate determinations showed complete hydrolysis of the potassium acid sulfate. The equilibrium value has not previously been determined by mustard-oil analysis.

The relative myrosin activity of plant tissue could be determined by measuring the amount of hydrolysis of a sinigrin substrate effected by a tissue macerate in a given time. To obtain the rate of mustard-oil liberation by the mustard-oil enzyme systems of resistant and susceptible plants, it was thought best to determine the allyl isothiocyanate liberated. For such a study it is necessary to allow the hydrolysis to proceed for a given time, then stop the reaction immediately without altering the concentration of the hydrolytic products, and determine one of the products (allyl isothiocyanate) by a chemical method that is not affected by the unhydrolyzed sinigrin or other products from the tissue macerate.

THE DETERMINATION OF ALLYL ISOTHIOCYANATE FROM THE HYDROLYSIS OF SINIGRIN

To develop a chemical method to serve this purpose, a comparison was first made of some of the methods that have been described for allyl isothiocyanate analysis of black mustard seed. Morvillez and Meesemeacker (*17*) have suggested the use of the absorption of iodine by the seed distillate as an estimation of allyl isothiocyanate, the absorption taking place at the double bond of the allyl group. Meesemeacker and Boivin (*16*) have used the iodine method directly on filtrates from seed macerates and thus have eliminated the tedious steam distillation. Although this procedure has been questioned (*11*), it was considered feasible and practical for use in these studies since it would permit the rapid determination of the allyl isothiocyanate immediately after stopping the enzymatic hydrolysis. The iodine absorption procedure as modified by Viebeck and Brecker (*29*) was found to be most practical and to give accurate and reproducible results when applied to the analysis of purified allyl isothiocyanate. The allyl isothiocyanate is converted to allylthiourea by excess ammonium hydroxide and the iodine absorption by the allyl group measured.

When this procedure was applied to the analysis of the hydrolytic products of a pure sinigrin substrate by a purified myrosin preparation, widely variable results were obtained which were much higher than the equilibrium value reported by Sandberg and Holly (*26*). These results suggested iodine absorption by the unhydrolyzed sinigrin, but when sinigrin was treated with iodine under these conditions, no iodine absorption could be observed. When sinigrin was first treated with excess ammonium hydroxide, as is done in these methods to convert allyl isothiocyanate to thiourea, and then treated with iodine, large but variable iodine absorptions were observed. When sinigrin was treated with a stronger base (0.1 N sodium hydroxide), a quantitative iodine absorption was obtained, and the solution gave a strong test for sulfate ions. No mustard oil could be detected in the alkaline solution.

It was necessary, therefore, to separate the allylthiourea from the hydrolysis mixture before estimation of the allylthiourea by iodine absorption. This was accomplished by ether extraction of the thiourea from the hydrolysis mixture in a continuous extractor after treatment with excess ammonium hydroxide. Iodine absorption then gave reproducible values which indicated that at equilibrium the myrosin had split off about 80 percent of the allyl isothiocyanate. The curve in figure 1 was obtained by measuring the allyl isothiocyanate released from a buffered (pH 7.2) solution of sinigrin by a fixed amount of a purified myrosin preparation. The sinigrin was prepared according to the method of Herissey and Boivin (*13*) and the myrosin by the method of Braecke (*5*).

DETERMINATION OF MYROSIN ACTIVITY OF ROOT TISSUE

Determinations of the relative myrosin activity of the root tissue of resistant and susceptible varieties of turnip and black mustard were made. With the turnip, separate determinations were made on the outer tissue, obtained by carefully peeling the fleshy root, and on the

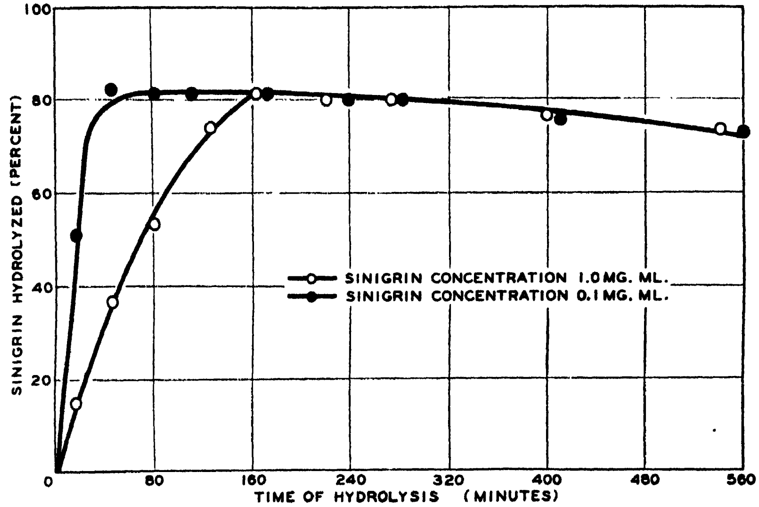


FIGURE 1.—The enzymatic hydrolysis of sinigrin as measured by allyl isothiocyanate formation.

remaining inner portion of the root. The fresh turnips were peeled, and 45 gm. of the outer and 90 gm. of the inner tissue were taken for study. The samples were sliced, then frozen in solid carbon dioxide and ground through a fine meat grinder while frozen. The tissue was allowed to thaw and ground further in a mortar with a little sand. This suspension was shaken for 12 hours with 75 ml. of a phosphate buffer at pH 7.2 and filtered.

Ten milliliter of the filtrate which would contain the water-soluble myrosin was added to 10 milliliter of a 1-percent sinigrin solution in phosphate buffer at pH 7.2. This mixture was maintained at 45° C. for 165 minutes. Ten milliliter of concentrated ammonium hydroxide was then added to stop the hydrolysis and the mixture heated for 1 hour at 70° C. to convert the mustard oil to the thiourea. The thiourea was extracted from the hydrolysis mixture by a 4-hour continuous extraction with ethyl ether. Twenty milliliter of water was added to the flask containing the ether extract and the ether evaporated by heating on a steam bath to about 60° C. The aqueous solution was neutralized to methyl red with dilute nitric acid and transferred to a 250–milliliter iodine titration flask. Enough distilled water was used in washing the extraction flask to bring the volume to about 100 milliliters. Then 10 milliliters of a solution of equal parts of 0.1 N HCl and glacial acetic acid was added. Five milliliters of 0.02 N standard iodine solution was added from a pipette, the flask stoppered, swirled, and allowed to stand in the dark for 30 minutes. The unused iodine was then determined by titration with 0.01 N standard thiosulfate. Blank analyses were made on solutions containing no added sinigrin to correct for iodine absorption by materials from the tissue extract. This absorption proved to be very low.

The determination of the myrosin activity of mustard roots was carried out in a similar manner, except that the fibrous nature of the roots necessitated a different technique in preparing the myrosin solution. The mustard roots were dried before a fan at 25° C., then ground to fine powder in a Wiley mill. A 2-gm. sample of this ground root tissue was ground further in a mortar with sand, then extracted for 12 hours by shaking with 50 milliliters of phosphate buffer. Ten milliliters of the filtrate was added to the buffered sinigrin substrate and the determination carried out as for the turnip tissue, except that the time for the enzymatic hydrolysis was changed.

The results obtained with a resistant and a susceptible turnip variety and with two resistant and two susceptible black mustards are shown in table 4. The activity is expressed as milligram of sinigrin hydrolyzed per gram (dry weight) of root tissue in the time indicated. A longer time was necessary with turnip tissue to obtain sufficient hydrolysis for accurate determination.

No correlation was found between myrosin activity and resistance to clubroot.

TABLE 4.—*Myrosin activity of black mustard and turnip root tissue*

Determination No.	Variety or collection	Resistance	Milligrams of sinigrin hydrolyzed per gram of root tissue (dry weight) in – 30 minutes	120 minutes	480 minutes
1[1]	Black mustard No. 3	Resistant	17.4	26.4	
	Black mustard No. 10	do.	11.6	23.7	
	Black mustard No. 8	Susceptible	11.0	24.2	
	Black mustard No. 14	do	13.4	17.3	
2[2]	Black mustard No. 3	Resistant	3.0	8.3	29.1
	Black mustard No. 14	Susceptible	3.3	5.5	18.1
			165	165	
			Outer tissue	Inner tissue	
3[3]	Turnip T-113	Resistant	11.6	5.5	
	Turnip 1-115-1x2B	Susceptible	23.6	5.0	

[1] Plants grown in greenhouse, entire root system used for analysis.
[2] Plants grown under field conditions, mature roots analyzed.
[3] Turnip roots were divided into outer tissue (largely periderm and phloem), and inner tissue (xylem), and each portion was analyzed separately. Time of hydrolysis 165 minutes in all cases.

DISCUSSION

This investigation was undertaken because it had been claimed that specific volatile mustard oils in certain crucifers were responsible for their resistance to the clubroot organism. The mustard oils which occur in the roots of turnip, cabbage, horseradish, black mustard, and white mustard were isolated and characterized. This study showed that the more commonly known mustard oil, allyl isothiocyanate, which occurs in the seeds of black mustard in the form of the glycoside sinigrin, is not found in the roots of any of these crucifers except horseradish. On the other hand, another mustard oil, β-phenethyl isothiocyanate, was found in the root tissue of each of the crucifers mentioned. Presumably this oil occurs also as a glycoside and is formed by hydrolysis of the glycoside by the proper enzyme system when the tissue is macerated. It has the same general degree of toxicity toward fungi as allyl isothiocyanate.

An intensive study was made of resistant and susceptible strains of black mustard and turnip. Quantitative estimation of the total amount of mustard oils in the roots of these plants failed to show correlation of isothiocyanate content to resistance or susceptibility to clubroot. This fact might be interpreted as showing either that the mustard oils, though toxic to the pathogen, were not responsible for resistance; or that the enzyme system in the susceptible plants did not release the free toxic oils from the nontoxic glycosides at the proper time or rate to be effective in imparting resistance to the tissue. A further study of this thioglucoside-splitting enzyme system in resistant and susceptible root systems showed no tangible relationship of enzyme concentration or activity to resistance or susceptibility.

It is thus evident that, although mustard oils that are highly toxic to fungus organisms, including the clubroot organism, may be obtained from the roots of many crucifers, they do not seem to inhibit infection or development of the pathogen in these tissues. Furthermore, they are not responsible in any demonstrable way for the resistance of certain varieties and strains to the clubroot organisms. An opinion is thereby reemphasized which has been previously expressed (*32*), viz, that the presence of a toxic substance in the tissue is not proof that it functions in the phenomenon of disease resistance.

SUMMARY

This investigation deals with the role of mustard oils in resistance to clubroot infection in the crucifers. A biochemical study has been made of the isothiocyanate content of root tissue as related to species and varietal resistance to invasion by *Plasmodiophora brassicae* Wor. β-phenethyl isothiocyanate has been isolated from root tissue of resistant and susceptible strains of turnip (*Brassica rapa*) and black mustard (*B. nigra*) and from susceptible strains of white mustard (*B. alba*) and horseradish (*Armoracia rusticana*). β-phenethyl isothiocyanate appears to be the principal mustard oil in the root tissue of many crucifers, both those susceptible and those resistant to clubroot.

Previous methods for estimating mustard oil were found to give variable recoveries when applied to the estimation of the isothiocyanate produced by the enzymatic hydrolysis of a mustard-oil glycoside. Improved analytical methods for estimating isothiocyanates were developed and applied to a study of (1) the enzymatic hydrolysis of sinigrin, (2) the isothiocyanate content of turnip and mustard root tissue as it relates to resistance to clubroot, and (3) the thioglucosidase (myrosin) activity of resistant and susceptible root tissues. Quantitative estimation of the total mostard-oil content and the relative myrosin activity of root tissues failed to show any correlation of isothiocyanate content or thioglucosidase activity to resistance or susceptibility to clubroot.

From these studies it appears that the mustard oils are not essential in enabling certain varieties of crucifers to prevent or retard clubroot development in their root tissues.

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EFFECT OF CHANGE OF TEMPERATURE ON RELATIVE TOXICITY OF ROTENONE AND PHENOL[1]

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INTRODUCTION

In toxicological studies conducted by the United States Department of Agriculture in which the goldfish (*Carassius auratus*) has been used as the test animal, the temperature regularly employed has been 27° C., as specified when this method of study was described (*6*).[3] This temperature may have to be lowered, however, because of the rapid development of bacterial diseases in fish stock kept at such a high temperature in the dechlorinated water that is now used. Consequently, experiments were undertaken in the hope of finding some orderly relationships that might permit correlation of comparisons at one temperature with those at another.

EXPERIMENTAL PROCEDURE

The test substances chosen were rotenone and phenol, because these had been used as standards of comparison in previous work and also because they differ greatly in type of toxic action, the former being toxic to goldfish at very low concentrations but relatively slow in action, and the latter requiring much higher concentrations and being much more rapid in action. The samples used were chemically pure. All test solutions of phenol were made from a 5-percent aqueous stock solution, but those of rotenone were made as usual from acetonic stock solutions. The highest concentration of rotenone showed a faint opalescence, indicating that the limit of solubility had been passed.

Toxicological tests were made on each compound at a constant temperature in the usual manner (*6*)—that is, survival time was determined at a number of concentrations—but in addition a third variable was introduced by repeating the tests at different temperature levels. The goldfish, a single lot obtained from one pond and weighing between 2 and 4 gm. each, were acclimated to laboratory conditions, having been held at 7° C. for 6 weeks, and were active and feeding well despite the low temperature. Tests were run at a constant temperature of 7°, and then the temperature of the stock

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[2] The writer is now with the Division of Control Investigations.

[3] Italic numbers in parentheses refer to Literature Cited, p. 80.

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tanks was raised slowly to 12° and held there for a week before the tests at this level were begun. The procedure was then repeated, the temperature being raised in 5° increments up to 27°, and the fish were acclimatized by holding them at each temperature a week before starting the tests at that level. This acclimatization has an important stabilizing effect on the survival time.

Since the elapsed time before testing, and therefore the age of the test animal, increases as the temperature is raised, any changes may be thought to be a function of age as well as temperature. The writer has found, however, that for a relatively long-lived organism, such as the goldfish, held under the conditions of these tests, such a variation in age causes no significant difference in toxicity measurements.

EXPERIMENTAL RESULTS

TOXICITY IN RELATION TO TEMPERATURE

The results of the tests are assembled in tables 1 and 2. A ready graphical comparison of the toxic action as expressed by these data is permitted by the survival-time and velocity-of-fatality curves in figures 1 and 2. The reciprocal nature of the two types of curves resulting from the use of the geometric mean allowed the use of intermediate points, obtained by interpolation, where they were of advantage in locating parts of the velocity-of-fatality curves.

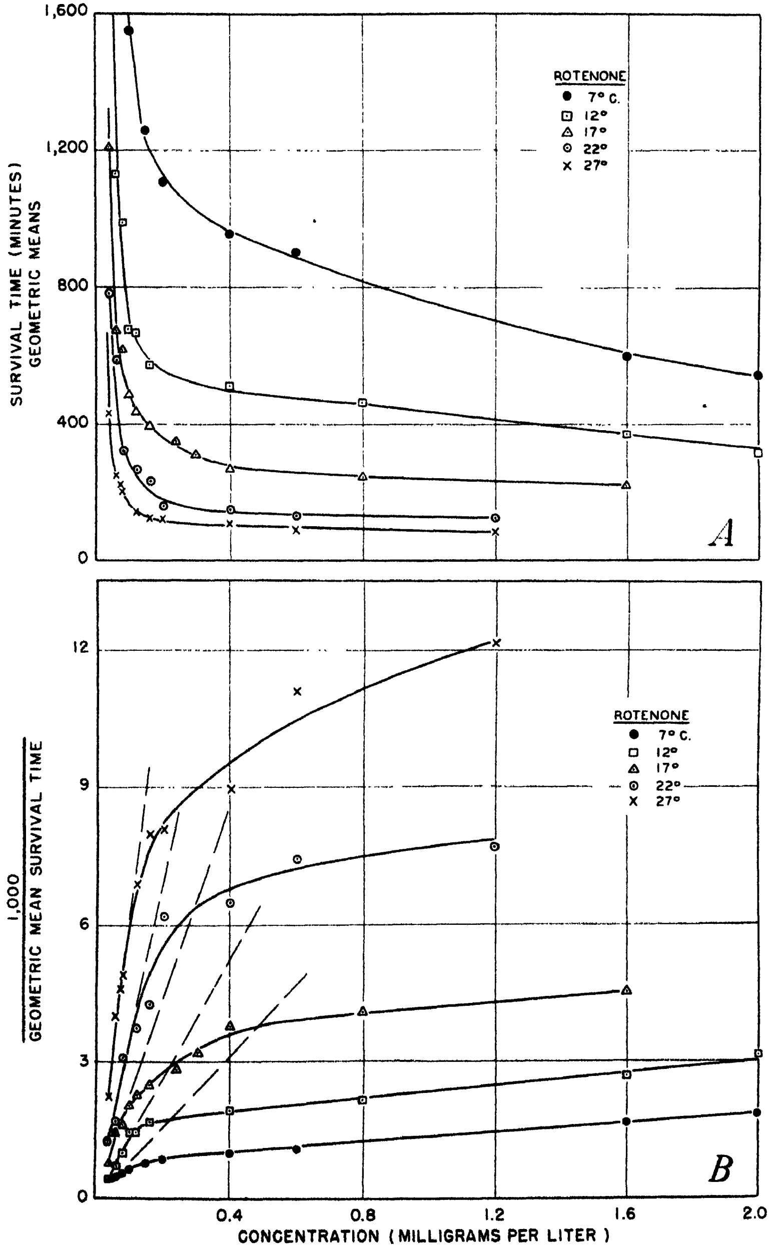

FIGURE 1.—Survival-time (*A*) and velocity-of-fatality (*B*) curves for rotenone.

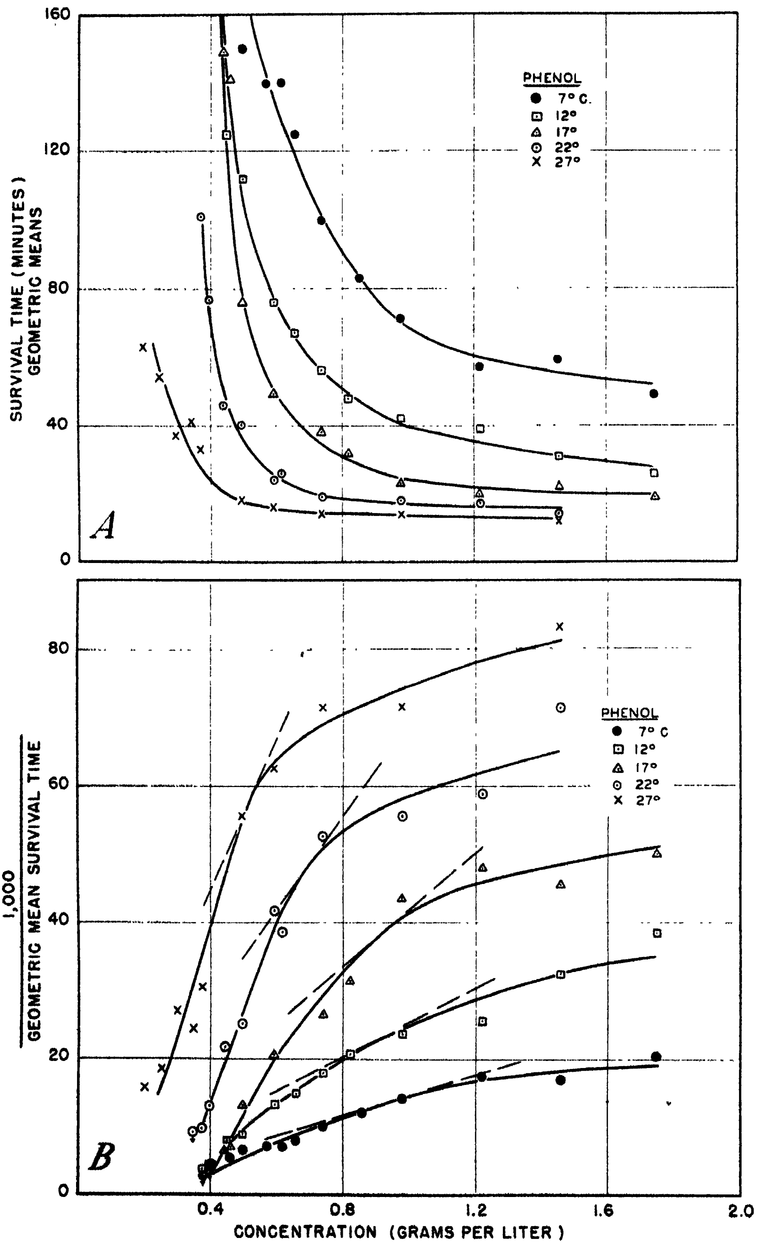

FIGURE 2.—Survival-time (*A*) and velocity-of-fatality (*B*) curves for phenol.

TABLE 1.—*Toxic action of rotenone on goldfish at different temperatures*

Temperature (°C.)	Concentration	Fishes used	Mean length of fishes	Mean[1] weight of fishes	Mean survival time[2]				1,000÷ geometric mean	Mean of 1,000÷ survival time
					Arithmetic	Geometric				
	Milligrams per liter	*Number*	*Millimeters*	*Grams*	*Minutes*	*Minutes*				
7.0±0.4	3.20	10	41	2.1	564±20	557		1.04	1.80	1.81
	2.00	14	42	2.3	541±12	537		1.02	1.86	1.87
	1.60	13	43	2.4	604±15	600		1.02	1.67	1.68
	.600	13	43	2.4	922±17	918		1.02	1.09	1.10
	.400	13	43	2.4	968±22	963		1.02	1.04	1.05
	.200	15	43	2.4	1,117±16	1,115	×÷	1.01	.897	.900
	.150	16	44	2.6	1,278±41	1,262		1.03	.792	.801
	.100	12	45	2.8	1,614±84	1,552		1.05	.644	.663
	.0800	12	44	2.6	1,883±116	1,801		1.06	.555	.574
	.0600	11	43	2.4	2,244±183	2,117		1.07	.472	.494
	.0500	12	45	2.8	2,340±174	2,210		1.07	.452	.474
	.0200	12	45	2.8	(3)					
12.0±0.4	2.00	12	43	2.4	330±17	318		1.05	3.14	3.24
	1.60	12	44	2.6	385±23	368		1.07	2.72	2.85
	.800	15	43	2.4	488±30	462		1.07	2.16	2.30
	.400	16	44	2.6	533±28	510		1.05	1.96	2.04
	.160	16	41	2.1	586±23	576	×	1.04	1.74	1.79
	.120	30	43	2.4	691±22	669	÷	1.03	1.49	1.54
	.096	16	45	2.8	709±41	675		1.05	1.48	1.53
	.0800	16	43	2.4	1,051+72	991		1.07	1.009	1.059
	.0600	16	42	2.3	1,191±80	1,131		1.06	.884	.901
	.0400	14	42	2.3	1,842±101	1,767		1.06	566	.587
	.0100	16	(4)	(4)	(5)					
17.0±0.3	1.60	16	43	2.4	223±7	220		1.03	4.55	4.61
	.800	16	42	2.3	248±8	244		1.03	4.10	4.18
	.400	15	43	2.4	275±13	264		1.04	3.79	3.94
	.300	17	43	2.4	319±15	309		1.05	3.24	3.34
	.240	17	43	2.4	365±18	351	×	1.05	2.85	2.96
	.160	17	42	2.3	414±22	398	÷	1.05	2.51	2.62
	120	20	42	2.3	461±25	437		1.06	2.29	2.40
	.100	20	43	2.4	512±29	485		1.05	2.06	2.16
	.0800	17	43	2.4	656±42	618		1.06	1.62	1.70
	.0600	18	42	2.3	725±46	678		1.06	1.47	1.55
	0400	16	45	2.8	1,360±134	1,209		1.05	.827	.923
	.0100	16	(4)	(4)	(6)					
22.0±0.3	1.20	15	43	2.4	128±5	126		1.04	7.94	8.15
	.600	15	44	2.6	143±11	133		1.06	7.52	7.95
	.400	14	43	2.4	153±5	150		1.04	6.67	6.76
	.200	13	43	2.4	168±10	161		1.06	6.21	6.45
	.160	17	44	2.6	245±13	235	×	1.05	4.26	4.44
	.120	17	43	2.4	284±16	268	÷	1.06	3.73	3.99
	.0800	18	43	2.4	344±19	323		1.06	3.10	3.33
	.0600	12	43	2.4	619±38	596		1.07	1.68	1.76
	.0353	17	45	2.8	855±60	784		1.07	1.28	1.38
	.0100	12	44	2.6	(7)					
27.0±0.2	1.20	14	43	2.4	83±2	82		1.03	12.2	12.4
	.600	15	43	2.4	90±3	90		1.03	11.1	11.3
	.400	13	43	2.4	112±4	111		1.03	9.01	9.13
	.200	18	41	2.1	128±6	123		1.04	8.13	8.33
	.160	16	40	2.0	130±7	125	×	1.05	8.00	8.25
	.120	15	41	2.1	148±4	145	÷	1.03	6.90	7.02
	.0800	16	43	2.4	223±19	203		1.09	4.93	5.42
	.0750	18	43	2.4	240±16	224		1.07	4.46	4.75
	.0600	15	42	2.3	262±15	249		1.06	4.02	4.22
	.0400	14	45	2.8	506±51	439		1.13	2.28	2.73
	.0100	12	(4)	(4)	(8)					

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[1] Estimated from length, which measurement excludes the caudal fin.

[2] The limits of error indicated are probable errors of the means. In the case of the arithmetic means they are differences from the means; in the case of the geometric means they are ratios to the means.

[3] 3 killed in 40 hours, 2 slightly affected; 7 apparently unaffected after 50 hours.

[4] The fishes were not measured but were of approximately the same size.

[5] 1 killed in 30½ hours; others apparently unaffected after 51 hours.

[6] Apparently unaffected in 51 hours.

[7] Apparently unaffected after 50 hours.

[8] Apparently unaffected after 36 hours.

TABLE 2.—*Toxic action of phenol on goldfish at different temperatures*

Temperature (° C.)	Concentration	Fishes used	Mean length of fishes	Mean[1] weight of fishes	Mean survival time[2]				1,000÷ geometric mean	Mean of 1,000÷ survival time
					Arithmetic	Geometric				
	Grams per liter	*Number*	*Milli-meters*	*Grams*	*Minutes*	*Minutes*				
7.0±0.3	1.75	10	43	2.4	50±2	49		1.04	20.4	20.7
	1.46	10	42	2.3	61±3	59		1.06	16.9	17.4
	1.22	10	42	2.3	59±3	57		1.05	17.5	18.0
	.980	12	40	2.0	73±3	71		1.04	14.1	14.4
	.855	12	44	2.6	85±5	83		1.06	12.0	12.5
	.739	10	42	2.3	103±7	100	×	1.07	10.0	10.4
	.655	14	43	2.4	129±7	125	÷	1.06	8.00	8.33
	.617	12	42	2.3	143±4	140		1.03	7.14	7.14
	.570	13	43	2.4	143±5	141		1.04	7.09	7.24
	.495	10	43	2.4	156±10	150		1.07	6.67	6.91
	.454	12	44	2.6	186±6	184		1.03	5.43	5.51
	.397	14	42	2.3	223±11	217		1.05	4.61	5.17
	.372	11	(3)	(3)	[4] >360	>360			<2.78	<2.78
12.0±0.3	1.75	10	44	2.6	27±1	26		1.04	38.5	38.8
	1.46	10	41	2.1	32±2	31		1.06	32.3	33.1
	1.22	11	43	2.4	40±2	39		1.04	25.6	26.2
	.980	10	44	2.6	43±2	42		1.05	23.8	24.6
	.820	11	45	2.8	50±3	48		1.05	20.8	21.4
	.739	13	41	2.1	59±3	56	×	1.06	17.9	18.7
	.658	12	43	2.4	69±4	67	÷	1.07	14.9	15.7
	.593	10	42	2.3	79±4	76		1.06	13.2	13.7
	.495	12	42	2.3	114±5	112		1.04	8.93	9.24
	.446	15	44	2.6	136±10	125		1.09	8.00	8.93
	.397	12	44	2.6	248±17	233		1.08	4.29	4.57
	.372	14	45	2.8	[5] 283±24	260		1.09	3.85	4.13
17.0±0.2	1.75	10	43	2.4	20±1	20		1.05	50.0	51.8
	1.46	11	44	2.6	22±1	22		1.06	45.5	48.0
	1.22	11	43	2.4	22±1	21		1.04	47.6	46.6
	.980	10	42	2.3	23±2	23		1.06	43.5	44.4
	.820	12	42	2.3	33±2	32	×	1.06	31.3	32.7
	.739	14	43	2.4	39±2	38	÷	1.06	26.3	27.8
	.593	11	42	2.3	51±2	49		1.05	20.4	20.8
	.495	10	42	2.3	84±7	76		1.10	13.2	15.1
	.461	14	40	2.0	181±26	141		1.14	7.09	8.56
	.441	15	43	2.4	167±14	149		1.09	6.71	7.58
	.397	27	(3)	(3)	[6] >325	>290			<3.44	4.0- 3.3
	.372	14	(3)	(3)	[7] >360	>300			<3.33	3.4- 1.9
22.0±0.2	1.46	10	42	2.3	14±1	14		1.05	71.4	74.2
	1.22	10	44	2.6	18±1	17		1.04	58.8	58.0
	.980	10	43	2.4	18±1	18		1.06	55.6	58.4
	.739	13	43	2.4	20±1	19		1.06	52.6	54.1
	.617	6	44	2.6	26±1	26	×	1.03	38.5	39.1
	.593	12	40	2.0	25±2	24	÷	1.06	41.7	43.6
	.495	19	43	2.4	44±3	40		1.09	25.0	27.7
	.441	13	42	2.3	50±4	46		1.10	21.7	23.8
	.397	15	45	2.8	82±5	77		1.06	13.0	13.5
	.372	24	43	2.4	122±12	101		1.10	9.90	11.8
	.346	15	42	2.3	[8] >204	>110			<9.09	11.1-10.5
27.0±0.2	1.46	10	41	2.1	12±1	12		1.04	83.3	86.7
	.980	10	43	2.4	14±1	14		1.04	71.4	75.0
	.739	12	41	2.1	14±1	14		1.04	71.4	74.5
	.593	13	41	2.1	17±1	16		1.07	62.5	67.7
	.495	14	40	2.0	20±2	18	×	1.09	55.6	59.8
	.372	14	42	2.3	35±3	33	÷	1.06	30.3	32.2
	.346	16	39	1.9	43±2	41		1.07	24.4	27.3
	.298	16	42	2.3	60±11	37		1.11	27.0	32.3
	.249	16	39	1.9	66±6	54		1.08	18.5	22.0
	.199	14	40	2.0	85±14	63		1.11	15.9	18.6
	.025	6	(3)	(3)	(9)					

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[1] Estimated from length, which measurement excludes the caudal fin.

[2] The limits of error indicated are probable errors of the means. In the case of the arithmetic means they are differences from the means; in the case of the geometric means they are ratios to the means.

[3] The fishes were not measured but were of approximately the same size.

[4] 27 percent killed in 8 hours.

[5] 86 percent killed in 8 hours.

[6] 63 percent killed in 8 hours.

[7] 50 percent killed in 8 hours.

[8] 67 percent killed in 8 hours.

[9] Slight effect but not lethal in 8 hours.

Since this work was designed to study toxicity well within the range of toxic concentrations, little information was obtained which bears on the difficultly determined thresholds of toxicity. It is obvious that within the range studied there is a decided increase in apparent toxicity with increase in temperature between 7° and 27° C.

QUANTITATIVE COMPARISON OF TOXICITY

For the comparisons of toxicity the measure proposed by the writer in 1935 (7) and used in subsequent work was adopted. This measure, the minimal product of concentration and survival time, ignores the tolerance parameters and considers toxicity only in the range of most efficient action with respect to both variables. Its value may be determined by calculation from the survival-time curve or graphically from the velocity-of-fatality curve by drawing the maximal tangent to the curve from the origin, as indicated with broken lines. In the latter case the value of the slope of this line is the reciprocal of the minimal product, and expresses the greatest ratio of dilution to survival time.

The data for comparison by this criterion are given in table 3. The minimal product of concentration and survival time is designated by $c_m t_m$ and, since toxicity varies inversely with this value, its reciprocal is also given. Its coordinates, c_m and t_m, are given so that the region approximating this condition may be located readily in the graphs. The ratio of increase of toxicity is based on the toxicity of phenol at 7° C. as unity. The value for $1/c_m t_m$ used as the base for the rotenone series was 8.12 liters per gram per minute, calculated with the aid of the mean value for relative toxicity, 0.00181. This procedure was used because the determination of the actual value for rotenone at 7° must have a relatively large error, owing to the difficulty of reading such long survival times and the use of the comparatively small number of fishes.

TABLE 3.—*Relative toxicity of rotenone and phenol at different temperatures*

ROTENONE

Temperature (°C.)	c_m	t_m	$c_m t_m$	Toxicity $\frac{1}{c_m t_m}$	Relative toxicity phenol to rotenone	Ratio of increase of toxicity	Relative effect of 5° increment
	Milligrams per liter	*Minutes*	*Gram-minutes per liter*	*Liters per gram per minute*	*Percent*		
7	0.045	2,500	0.113	8.85		1.09	...
12	.080	910	.0728	13.7		1.69	1.55
17	.070	600	.0420	23.8		2.93	1.73
22	.080	350	.0280	35.7		4.40	1.50
27	.070	223	.0156	64.1		7.89	1.80
PHENOL							
7	1,000	68	68.0	0.0147	0.166	1.00	...
12	900	44	39.6	.0253	.185	1.72	1.72
17	900	25	24.0	.0417	.175	2.84	1.65
22	660	21	13.9	.0719	.201	4.89	1.72
27	520	17	8.84	.113	.176	7.69	1.57
Mean of all ratios					.181		1.66

STATISTICAL ANALYSIS OF CORRELATION BETWEEN RELATIVE TOXICITY AND TEMPERATURE

Because of the nice relationships found in the foregoing study of the freehand curves, it is desirable to go beyond the mere determination of an average of variation of the mean survival times and seek information on the precision of the minimal *ct* measurements.

If the minimal *ct* measurements are estimates of a parameter defining a characteristic of this population, there should be some orderly relationship between the relative toxicities of the two compounds at different temperatures. If such a relationship is sought between two substances of such different mode of action, it seems logical to exclude from the statistical comparison the extremes of action, where the two variables vary so disproportionately. To the extreme left in the direction of diminishing concentrations, the usefulness of time measurements decreases until at the thresholds the comparison can be one of concentration alone and the ratio of relative toxicity is expanded its widest in favor of the compound evincing greater toxicity from the concentration aspect—in this case rotenone. To the extreme right in the direction of increasing concentrations, the usefulness of concentration measurements decreases until the comparison may become one of time alone, and if one of the compounds is a relatively fast-acting toxicant, as is phenol in this case, not only may the ratio vary greatly but even the order of toxicity may be reversed. To bring into consideration the spheres of such adverse influences will at least weaken the stability of the parameter to be estimated by this criterion. Therefore, in each case the concentrations selected for statistical treatment from the data in tables 1 and 2 are in a range which corresponds to as many points on the curve as affect the location and evaluation of the minimal *ct* point without bringing in the adverse effects of the extremes. This range was judged to be delimited by the use of 140 percent of the $c_m t_m$ values (table 3) as the maximal product of concentration and survival time.

To evaluate the sampling errors of the $1/c_m t_m$ estimations, curves that might fit the variates were studied. A semilog curve, obtained with the use of logarithms of concentrations and reciprocals of survival times, was found to be well adapted to such treatment. Straight lines were fitted by the method of least squares to the data within the stated range of concentrations. These regression lines are drawn and the complete semilog curves indicated in figure 3. The regression equations are given below. For rotenone X is 1,000 times the concentration in milligrams per liter; for phenol X is 100 times the concentration in grams per liter. For both compounds Y is 1,000 divided by the survival time in minutes.

Temperature (° C.)	For rotenone	For phenol
7	$Y = -0.7646 + 0.7152 \log X$	$Y = -41.04 + 27.56 \log X$
12	$Y = -2.748 + 2.068 \log X$	$Y = -75.14 + 50.02 \log X$
17	$Y = -3.623 + 2.864 \log X$	$Y = -109.2 + 74.13 \log X$
22	$Y = -8.241 + 5.994 \log X$	$Y = -151.4 + 107.5 \log X$
27	$Y = -10.964 + 8.539 \log X$	$Y = -206.3 + 153.1 \log X$

The minimal products are given by those tangents to the curves expressed by these equations which pass through the origin; their values are determined with the use of the knowledge that these tangents are the first differential coefficients of the curves.

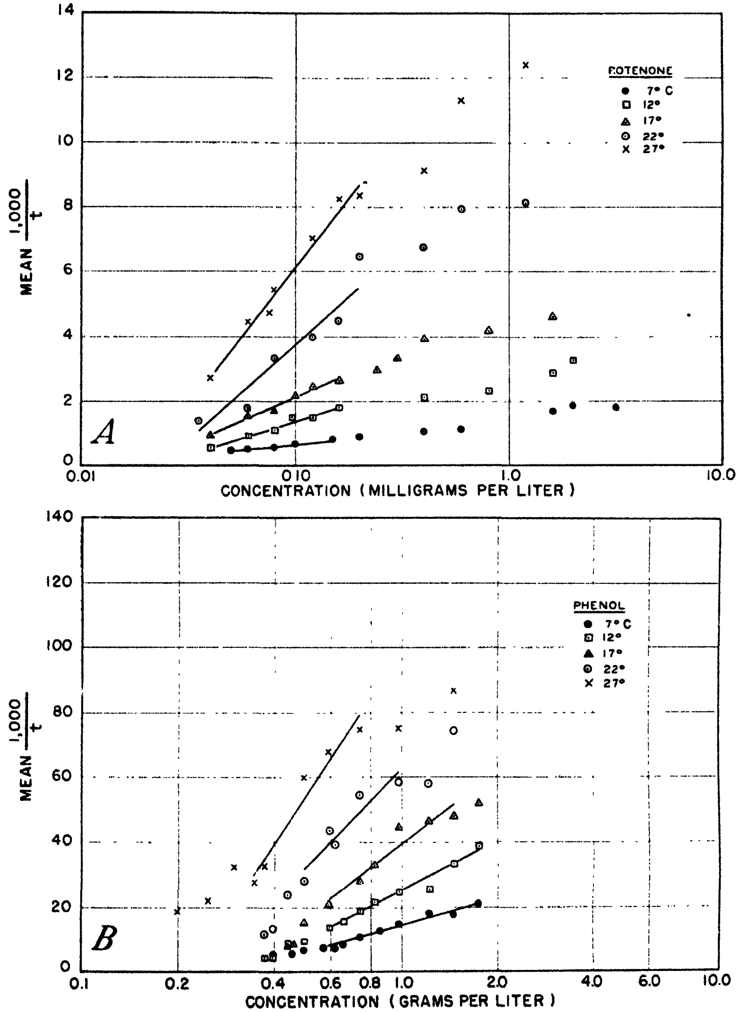

FIGURE 3.—Regression of reciprocal of time on logarithm of concentration for rotenone (*A*) and phenol (*B*) at different temperatures.

Since the curves are completely specified by two factors, slope and position, the error estimates of each factor will contribute to the error of the minimal *ct* products. The latter may be calculated in two ways[4]: (1) From the method of extremes, using a sampling range of ±1 standard error for each factor and combining them; or (2) from the equation

$$S \text{ (in percent)} = \frac{230.3\sqrt{S_{\bar{y}}^2 + (\bar{x} - \log c_m)^2 . S_b^2}}{b}$$

in which S denotes the standard error of the reciprocal of $c_m t_m$; $S_{\bar{y}}$, the standard error of the mean reciprocal of survival time; $\bar{x}$, the mean log concentration; c_m, the concentration corresponding to the minimal *ct;* S_b, the standard error of the regression coefficient, b. S is actually the relative error of $1/t_m$, but since all the error is derived from the determination of the value of this variate, it may be regarded as the relative error of the reciprocal of $c_m t_m$. The first method also permits ready estimation of the sampling variation of location of the minimal *ct* product. The two methods gave practically identical results, and the second was, in fact, used throughout as a check.

The results of this statistical analysis are given in table 4. The ratios for increase of toxicity were calculated in the same manner as for table 3. In this case, however, the base for the rotenone series is 9.038 liters per gram per minute.

It is apparent that, insofar as the actual ratios are concerned, there is little choice between the method of freehand curves based on geometric mean survival times and concentration and the statistical method of linear regression computed from reciprocals of survival time and logarithm of concentration. There is a definite and regular increase, though small, in the $1/c_m t_m$ values for rotenone obtained by the latter method over those obtained by the former, because in such distributions the reciprocal of the harmonic mean is always slightly larger than the reciprocal of the geometric mean, and the difference near the critical point is relatively greater in the case of rotenone. This difference results in a slightly lower mean (0.16 percent) for the toxicity of phenol as compared with that of rotenone, than is obtained with the use of geometric means (0.18 percent). The statistical method produces ratios showing slightly more regular effects of increase in temperature. The errors for the minimal *ct* products estimated by this method show the phenol values to be determined with more precision than those for rotenone; those for the former vary from 2.5 to 4.2 percent whereas those for the latter vary from 5.0 to 7.4 percent from 12° to 27° C. and jump to 14.3 percent at the 7° level. The determination in the last instance was difficult because of the necessity for reading extremely long survival times, and the location of the $c_m t_m$ point appears to be, in a sense, slightly extrapolative as recorded.

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[4] The equation was developed by C. M. Smith and the other method was suggested by F. M. Wadley, both of this Bureau. Grateful acknowledgment is also made for other helpful suggestions by them in the statistical treatment of the data.

Table 4.—*Statistical analysis of the effect of temperature on the relative toxicity of rotenone and phenol when measured by the minimal ct product*

ROTENONE

Temperature (°C.)	c_m	t_m	$c_m t_m$	Toxicity $\frac{1}{c_m t_m}$	Relative toxicity phenol to rotenone	Ratio of increase of toxicity R_T	Relative effect of 5° increment Q_5	Relative effect of 10° increment Q_{10}
	Milligrams per liter	*Minutes*	*Gram-minutes per liter*	*Liters per gram per minute*	*Percent*			
7	0.03187±0.00861	3,219	0.1026±0.01471	9.747±1.46		1.08		
12	.05798± .00713	1,114	.06459± .00325	15.48± .79		1.71	1.59±0.24	
17	.05003± .00840	804	.04022± .00297	24.86±1.86		2.75	1.61± .14	2.55±0.41
22	.06443± .00884	384	.02475± .00159	40.40±2.61		4.47	1.63± .16	2.61± .21
27	.05226± .00764	270	.01409± .00097	70.97±4.95		7.85	1.76± .17	2.85± .29
PHENOL								
7	838.3± 57.7	83.5	70.03±1.74	0.01428±0.00036	0.147±0.021	1.00		
12	864.1± 79.4	46.0	39.77±1.18	.02514± .00075	.162± .009	1.76	1.76±0.07	
17	807.6±105.0	31.1	25.08± .92	.03987± .00147	.160± .013	2.79	1.59± .07	2.79±0.12
22	696.2±109.3	21.4	14.91± .61	.06707± .00273	.166± .013	4.70	1.68± .09	2.67± .13
27	605.0± 56.8	15.0	9.10± .38	.1099 ± 00458	.155± .013	7.70	1.64± .09	2.76± .15
Mean of all ratios					.158±0.0064		1.66±0.05	2.71±0 10

DISCUSSION

Two conclusions can be drawn at once from the results. Despite the great dissimilarity in the toxic action of rotenone and phenol, when measured by this criterion the increase in toxicity with increase in temperature is proportionately the same for each compound, and the ratio of increase is the same for each 5° increment between 7° and 27° C. It follows that rotenone and phenol have the same relative toxicity when measured at any temperature level in this range. The geometric increase in relative toxicity with arithmetic increase in temperature is shown in figure 4. The equation for both compounds, determined by the method of least squares, is

$$R_T = 0.515e^{0.100T}$$

in which R_T is the ratio of toxicity at temperature T° to that at 7°. When the values are expressed in liters per gram per minute, the specific equations for toxicity at temperature T° are, for rotenone,

$$\left(\frac{1}{c_m t_m}\right)_T = 4.66e^{0.100T}$$

and for phenol,

$$\left(\frac{1}{c_m t_m}\right)_T = 0.00736e^{0.100\,T}.$$

Thus, for each compound, the logarithm of the value for toxicity is a linear function of the temperature.

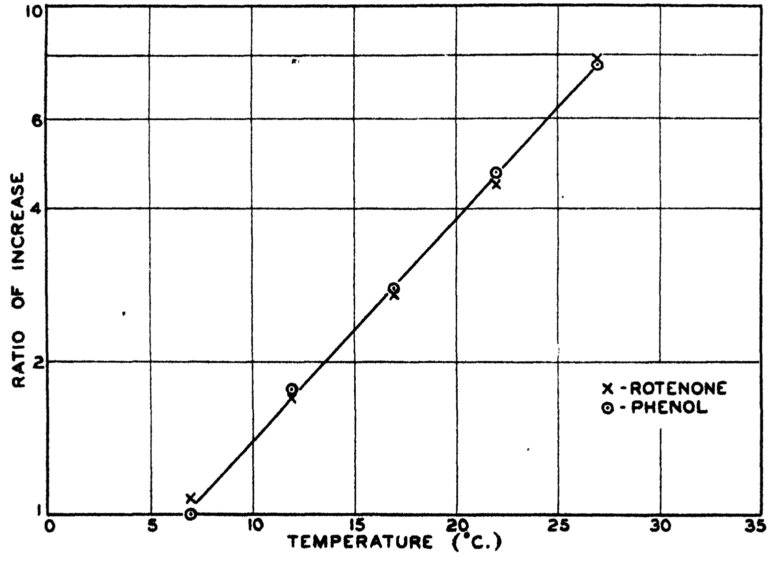

Figure 4.—Increase in toxicity of rotenone and phenol with increase in temperature in the range 7° to 27° C.

If relative toxicity as measured by this method is independent of the temperature at which comparisons are made, the temperature effects found above must be due solely to acceleration of one or more physiological processes in the test animal. In this case the controlling factor would most likely be a respiratory phenomenon. To make comparisons with temperature coefficients for respiratory processes reported in the literature (*2, 4, 5, 9, 10, 11*), the ratio of increase of effect for a 10° C. increase in temperature (Q_{10}) was calculated for the two substances, as shown in table 4.

It may be noted that the increase in toxicity is 2.7-fold for a rise of 10°, and is so nearly constant that we most likely have to do here with a simple and not a complex reaction. This value is about the usual ratio of increase in the velocity of a chemical reaction with rise in temperature. In fact, the results obtained over the temperature range given agree well with the relation between the rate of a chemical reaction and temperature as expressed by Van't Hoff's equation (*8*)

$$V_1 = V_0 e^{c(t_1 - t_0)}$$

where V_1 and V_0 are the velocities of the reaction at temperatures t_1 and t_0, respectively. The improved modification of this equation making use of absolute temperature and the gas constant, the Arrhenius equation (*1*),

$$V_1 = V_0 e^{\frac{\mu}{2}\left(\frac{t_1 - t_0}{t_0 t_1}\right)}$$

is reported to express more accurately the effect of temperature on many biological reactions (*3, p. 270*). This equation does express the data nearly as well for phenol with a range of values for the thermal characteristic, μ (determined for the four 5° increments), of 15,100 to 17,900 and a mean of 17,000 calories per mole. Although for rotenone there is a slight increase in Q_5 with increase in temperature (table 4) which is reflected in the slight concavity upwards of a curve through the rotenone points in figure 7, these differences are obviously not significant. However, since the trend is in the reverse direction to the condition for which the Arrhenius modification is a correction, the range of μ values for rotenone when this equation is applied becomes relatively greater, 14,700 to 19,800, with a mean of 16,600 calories per mole. Calculated from the equation of the curve in figure 4, the range of values for μ for the same 5° increments is from 15,800 to 17,600, with a mean of 16,900 calories per mole.

In this connection it may be mentioned that Ege and Krogh (*5*), studying the effect of temperature change on oxygen consumption (measured in cubic centimeters per kilogram per minute) of a single 9.3-gm. goldfish, reported results that were practically the same whether or not the fish was narcotized with urethane. Q_{10} was found to be approximately 2.7 in the temperature range 5° to 28° C. If calculated according to the Arrhenius equation, μ is found to be about 16,000 calories per mole. However, Ege and Krogh concluded that, since there was a steady and considerable decrease in Q_{10} from 0° to 28° C., Van't Hoff's temperature coefficient did not express the relationship satisfactorily.

Wells (*11*) came to the same conclusion after studying the relationship between temperature and oxygen uptake but with the Pacific killifish (*Fundulus parvipinnis*) as the test animal.

Sumner and Doudoroff (*10*), using the goby (*Gillichthys mirabilis*) and taking the rate of death from each of two lethal agents, potassium cyanide of a single concentration (0.001 molar in sea water) and boiled sea water, as a measure of respiratory metabolism, reported that the reciprocal of the survival time varied logarithmically with the temperature in the range 10° to 30° C., Q_{10} lying between 2.6 and 2.8 with the first agent and, with much less constancy, between 2.3 and 3.0 with the second.

Crozier and Stier (*4*) reported that the temperature characteristic for the frequency of rhythmic opercular movements in six quiescent goldfish, averaging 60 mm. in length (probably over-all), was 16,500 calories.

Bělehrádek (*2*), after a thorough survey of the work prior to 1930 on the use of temperature coefficients in connection with biological phenomena, considered the application of chemical temperature-velocity formulas to have failed.

Study of the application of these two formulas to the data obtained by the writer has emphasized a fundamental consideration that is usually ignored. Q_{10} is constant for both compounds when toxicity is measured by the minimal product, $c_m t_m$, and is practically constant when velocity is measured by $1/t$ at any one concentration near c_m. However, Q_{10} gradually tends to increase with increase in temperature if velocities are measured at concentrations below c_m but, on the other hand, to decrease with increase in temperature if measured at higher concentrations. The results of work by Powers (*9*) on the effect of temperature change on the toxicity of lithium chloride to goldfish (weight 1 to 2 gm.), though of an approximate nature, agree with the first half of this statement. Whether measured by survival time or by the slope of the theoretical velocity-of-fatality curve (the straight line approximating that part of the velocity-of-fatality curve near its point of inflection), or whether calculated from a temperature-relative toxicity curve, increase of Q_{10} with rise in temperature is indicated. The range of concentrations studied is at the left of the concentration that would correspond to a minimal *ct* product; that is, the product (within experimental error) increases with decrease in concentration. Moreover, comparisons by the theoretical velocity-of-fatality curve will always be comparisons at concentrations to the left of that of the minimal *ct* product.

When velocities are compared at some concentration other than that of the minimal *ct* product, this type of instability for Q_{10} is a natural condition for such sigmoid curves as complete velocity-concentration curves if they converge as they approach the limiting tolerance factors. The values for the thermal increment, μ, would vary with the concentration, although in this study the variation is slight. Therefore, just as the comparison of isotherms describing biological reactions in the dimensions time and concentration required study of the effect of change of temperature, so the comparisons of reactions in the dimensions time and temperature require study of the effect of change in concentration. Thermal increments are usually reported without explanation as to why that particular concentration was used; μ may or may not be the same at some other concentration.

It appears, then, that of the physiological reactions affected by change in temperature the main or controlling effect is on the respiratory processes of the test animal. In consequence, variation of apparent toxicity with change in temperature seems to be due solely to a response of the test animal; relative toxicity as inherent in the nature of the compound, when measured by this method, is the same regardless of the temperature level at which measured, provided, of course, that the limits of the range are not so extreme as to bring in other influences. In other words, relative toxicity, as shown by rotenone and phenol with this method, is independent of the temperature level at which measured.

CONCLUSIONS

(1) The use of the minimal product of concentration and survival time as a criterion of relative toxicity measures a parameter of the test population with respect to these two variables with such definiteness that introduction of another variable, such as temperature, does not affect the value obtained for relative toxicity with that particular population. This does not mean that all goldfish of the same size and age will give the same value as found above, for there is evidence from previous work by the author that fish from different ponds and of different ancestry give a different value. But there is also evidence that fish of uniform size and age from any one pond and of the same ancestry, even though taken from populations in different years, give values not greatly different. The value of relative toxicity thus has a stability that has not been shown to be obtainable with other measures.

(2) Under the conditions of these tests relative toxicity may be defined as a function of the nature of chemical compounds and its values are not affected by change of temperature. Such a change influences only the physiological processes of the test animal, and the apparent effect on toxic action is a function of the animal's resistance. In this sense toxicity and resistance do not have a simple inverse relationship but are distinct conceptions, the former inherent in the nature of the toxicant and the latter inherent in the nature of the test animal.

SUMMARY

The effect of temperature on apparent toxicity, when measured by the criterion of the minimal product of concentration and survival time, has been studied, with rotenone and phenol as the test materials and small goldfish as the test animals. The experiments were conducted at temperatures ranging from 7° to 27° C. in 5° increments. All results were subjected to statistical analysis.

Despite the great dissimilarity in the mode of toxic action of rotenone and phenol, increase in toxicity with increase in temperature, when measured by this criterion, was found to be proportionately the same for the two compounds. Phenol was 0.16 percent as toxic as rotenone at any temperature in this range.

The ratio of increase was found to be the same for equal increments in temperature, 1.66 for a 5° increment.

The geometric increase of relative toxicity (R_T) with arithmetic increase in temperature (T° C.) is summarized by the equation

$$R_T=0.515e^{0.100T}.$$

When expressed in liters per gram per minute, the value of toxicity at T° C. for rotenone is $4.66e^{0.100T}$, and for phenol is $0.00736e^{0.100T}$.

Toxicity and resistance do not have a simple inverse relationship but are distinct conceptions, the former inherent in the nature of the toxic compound and the latter inherent in the nature of the test animal.

Resistance only is affected by change in temperature. Relative toxicity is a function of the chemical compound and remains the same at any level within a range of temperatures not deleterious to the test animal.

The criterion of relative toxicity, the minimal product of concentration and survival time, therefore measures a definite characteristic of the test population.

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RELATION BETWEEN PARASITIZATION OF TWIG-INFESTING LARVAE OF THE ORIENTAL FRUIT MOTH AND SUBSEQUENT INFESTATION OF RIPE PEACHES[1] [2]

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INTRODUCTION

Information on the effect of parasites in reducing the infestation of the peach crop by the oriental fruit moth (*Grapholitha molesta* (Busck)) is important for determining the advisability of continued widespread liberations of these parasites. In this study an attempt has been made to show whether a relation can be detected between parasitization[3] in twigs at the peak of second-brood infestation and the total moth infestation of ripe peaches during the harvesting period for Elberta and other varieties ripening at about the same time. In the section of the country in which these observations were made, second-brood infestation of twigs usually occurs from the latter part of June until about the middle of July, and ripe-fruit infestation of Elberta peaches from about the first of August to the first week in September.

REVIEW OF LITERATURE

One rather intensive study of the relation between parasitization and fruit infestation was made by Daniel[4] in Niagara County, N. Y., from 1928 to 1931, inclusive. Daniel noted that fully one-third of the fruit infestation was not visible externally, and the data in his paper indicate that such injury ranged from less than 40 percent to more than 300 percent of the visible injury. He commented that this condition makes valueless any counts that do not take it into consideration and concluded that a large portion of the observed reduction in fruit infestation was caused by parasites. This work in Niagara County was continued during 1932 and 1933, and in other reports by Daniel and coworkers[5] [6] the conclusions were essentially the same as previously noted.

In 1940 Yetter and Allen[7] reported a study of larval parasitization and ripe-fruit infestation in eight orchards in Burlington County,

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[1] Received for publication November 24, 1942.

[2] Many individuals had a part in accumulating the data used in this paper. Information for the New Jersey orchards was collected by M. H. Brunson and W. P. Yetter; the rearing of twig collections for the record of parasitization was done by G. J. Haeussler; the field work in Virginia, West Virginia, and Maryland and the assembling of the data were done with the assistance of E. L. Plasket and D. W. Clancy.

[3] The term "parasitization" as used in this paper signifies parasitization of twig-infesting larvae of the second brood of the oriental fruit moth. A number of species of parasites were involved, but the most important species was usually *Macrocentrus ancylivorus* Roh.

[4] DANIEL, D. M. MACROCENTRUS ANCYLIVORUS ROHWER, A POLYEMBRYONIC BRACONID PARASITE OF THE ORIENTAL FRUIT MOTH. N. Y. (Geneva) Agr. Expt. Sta. Tech. Bul. 187, 101 pp., illus. 1932.

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N. J. In the group of orchards having the higher rates of parasitization the average infestation was much less than in the group having the lower rates. In the same year Brunson[8] published the results of mass liberation experiments in Mercer County, N. J., in which he made intensive observations on the parasitization and moth population in five orchards. These experiments also indicate that high parasitization is usually associated with low fruit infestation.

A few other workers have made observations on the relation of parasitization to fruit infestation, but most of these observations have been based on total emergence of relatively small numbers of insects, or on fruit counts in which the highly variable portion of the total injury invisible externally was not accurately determined by cutting fruit samples.

EXPERIMENTAL DATA AND ITS SOURCE

The relation between parasites and fruit infestation is thus seen to be essentially one of the interrelation of populations. To express fully this relationship in any given orchard it would be necessary to obtain information on the weekly fluctuations in parasite and moth populations per unit area. It would also be necessary to measure the effect of various other factors, such as unfavorable weather, cocoon and egg parasites, predators, unfavorable twig growth, lack or scarcity of fruits, control measures used by the grower, changes produced by migration to or from the orchard—considering them all in relation to the moth population at the beginning of the season. It is obviously impossible in the conduct of any small project to obtain such complete information for a large number of orchards. However, if the sum of all the unmeasured factors affecting the population of the fruit moth tends to be so small as to be overshadowed by the factor of parasitization, or, if not small, shows some degree of uniformity for orchards over a certain area or during a certain season, the true effect of such parasitization will become apparent without adjustment for these other factors. The study reported here has been based on this assumption.

The data used in this study are the records from the same orchards and for the same seasons as were used by Yetter and Allen[9] and Brunson[10], and, in addition, from a larger series of orchards, including 11 peach-growing districts, extending from Lovingston, Va., to Princeton, N. J., for the 3 years 1937 to 1939, inclusive. In the New Jersey orchards parasitization was determined from large numbers of infested twigs collected over 4-day periods, as described by Yetter and Allen. In the other orchards the parasitization was determined from two collections of twigs in each orchard at or near the peak of infestation for the second brood of fruit-moth larvae. The fruit records were based on samples of 300 to 400 peaches taken from each orchard at harvesttime, all of which were cut open. The data differ in one important respect from those obtained by Daniel and his associates—that is, they provide a basis for the comparison of different orchards in any year—whereas Daniel's comparisons are chiefly of the differences during several successive years for one district.

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[8] BRUNSON, M. H. MASS LIBERATIONS OF PARASITES OF THE ORIENTAL FRUIT MOTH FOR IMMEDIATE REDUCTION OF INFESTATION. Jour. Econ. Ent. 33:346-349. 1940.

[9] See footnote 7.

[10] See footnote 8.

These observations, which include 87 summations of orchard parasitization and fruit infestation obtained from 51 orchards, are presented in table 1, and furnish the data upon which the subsequent comparisons are made.

TABLE 1.—*Surveys of parasitization of second-brood larvae of the oriental fruit moth in infested twigs and the injury to ripe peaches, 1937–39*

District	Orchard No.	Fruit moth larvae reared from samples	Larvae parasitized	Bearing trees per acre	Fruits harvested per tree	Fruits injured			
						Relative injury		Fruits per tree	
						New	Total	New	Total
1937 SURVEY									
		Number	*Percent*	*Number*	*Number*	*Percent*	*Percent*	*Number*	*Number*
Virginia:									
Lovingston	1	187	56.7	106	728	5.3	8.3	39	60
	2	87	4.6	139	779	3.5	6.5	27	51
	3	83	8.4	128	587	4.3	6.8	25	40
	7	45	82.2	49	678	2.5	3.3	17	22
Staunton	8	47	2.1	91	365	12.3	15.3	45	56
	9	55	5.5	75	378	11.0	14.0	42	54
Harrisonburg	11	337	5.9	101	54	37.8	46.3	20	25
Timberville	15	229	9.0	59	841	7.0	9.0	59	76
	16	76	1.3	98	742	3.3	3.8	25	28
	17	70	0	118	596	4.5	5.5	27	33
Clear Brook	19	29	0	92	655	2.5	4.0	16	26
	20	111	2.7	37	326	22.5	31.3	73	102
Maryland:									
Smithsburg-Ringgold	30	274	55.8	161	901	1.7	2.1	16	19
	31	49	69.4	134	126	2.5	6.0	3	8
	33	47	63.8	69	1,411	2.3	3.3	32	47
	35	595	46.9	119	612	6.0	8.6	37	53
New Jersey:									
Moorestown	40	1,767	71.0	102	1,904	9.9	12.2	188	232
1938 SURVEY									
Virginia:									
Crozet-Afton	4	67	95.6	91	583	1.3	4.0	8	24
	6	56	96.4	104	631	1.3	2.0	8	12
Staunton	8	93	15.1	39	200	12.0	19.5	24	40
	9	153	5.2	83	92	28.6	42.0	26	40
Harrisonburg	11	85	12.9	101	400	37.6	47.3	150	189
	12	71	25.4	53	619	22.0	26.6	136	165
Timberville	14	135	48.9	70	118	22.0	34.6	26	41
	15	111	27.0	101	683	27.2	32.2	187	222
	16	92	32.6	133	355	12.8	14.5	47	53
Clear Brook	20	36	13.9	30	137	10.0	12.2	14	17
	21	17	29.4	105	313	7.7	9.7	27	34
West Virginia:									
Martinsburg	23	131	58.8	95	418	9.8	15.0	42	65
	24	128	46.1	38	533	17.0	26.0	87	136
	25	134	88.8	107	957	7.5	7.5	71	71
Maryland:									
Hancock	27	75	42.7	109	274	6.2	14.2	17	39
	28	63	93.7	78	195	1.3	1.6	2	3
	29	60	90.0	132	293	2.7	3.7	8	11
Smithsburg-Ringgold	30	32	65.6	152	407	1.7	1.7	8	8
	32	70	98.6	120	1,004	.6	.6	6	6
	34	3	0	41	125	6.0	7.2	8.	10
New Jersey:									
Moorestown	36	283	57.2	58	349	21.2	27.2	80	100
	37	1,181	60.1	75	367	27.5	31.0	104	117
	38	210	63.2	85	225	2.5	3.7	5	8
	41	467	86.1	76	394	3.5	4.7	15	20
	42	132	88.9	103	411	2.3	3.0	10	13
	43	952	72.7	78	436	11.8	21.0	51	93
	44	436	77.4	112	227	15.0	17.5	34	40
	45	679	84.5	90	139	9.2	16.2	13	23
Mercer County	47	574	84.8	102	75	19.0	26.2	14	20
	48	782	83.1	90	553	4.5	5.2	25	30
	49	3,268	59.6	86	387	15.0	17.5	58	69
	50	108	85.5	109	163	10.2	10.7	17	18
	51	251	79.0	107	660	21.7	23.2	147	158

TABLE 1.—*Surveys of parasitization of second-brood larvae of the oriental fruit moth in infested twigs and the injury to ripe peaches, 1937–39*—Continued

1939 SURVEY

District	Orchard No.	Fruit moth larvae reared from samples	Larvae parasitized	Bearing trees per acre	Fruits harvested per tree	Fruits injured			
						Relative injury		Fruits per tree	
						New	Total	New	Total
Virginia:		*Number*	*Percent*	*Number*	*Number*	*Percent*	*Percent*	*Number*	*Number*
Lovingston	1	220	12.7	100	100	44.7	50.7	45	51
	3	215	8.8	134	753	18.1	18.4	136	138
Crozet-Afton	4	166	16.3	94	888	3.8	3.8	34	34
	5	181	11.6	106	583	4.1	4.8	24	28
	6	124	23.4	105	558	1.0	1.3	6	7
Staunton	10	221	48.0	78	460	6.0	7.7	27	35
Harrisonburg	11	241	2.9	101	311	32.2	39.2	100	122
	12	147	8.8	53	280	22.2	29.9	62	84
Timberville	13	257	16.0	106	294	14.7	17.4	43	51
	16	215	3.7	132	218	7.5	9.1	16	20
	18	216	3.7	102	490	18.9	22.7	92	111
Clear Brook	20	197	43.1	41	321	5.4	6.4	17	21
	21	176	25.6	32	582	12.3	14.2	71	82
	22	157	68.8	100	448	1.3	2.3	6	11
West Virginia:									
Martinsburg	23	168	81.0	97	1,102	2.3	2.3	25	25
	25	178	81.5	103	1,173	1.3	1.7	15	20
	26	202	57.9	60	1,162	1.3	2.7	15	31
Maryland:									
Hancock	27	165	84.8	111	237	1.1	1.1	2	3
	28	24	72.4	76	591	0	0	0	0
	29	1	100.0	139	309	.7	.7	2	2
Smithsburg-Ringgold	30	30	46.7	171	888	2.7	2.7	24	24
	32	31	80.6	126	626	.3	.8	2	5
	34	18	61.1	44	166	9.3	9.3	15	16
New Jersey:									
Moorestown	36	69	47.8	58	556	....	21.3	....	118
	37	258	46.9	61	497	....	34.5	....	171
	38	409	85.8	81	669	....	3.9	....	26
	39	482	90.0	138	419	....	12.7	....	53
	41	454	82.2	72	543	....	5.8	....	31
	42	250	58.4	105	124	....	15.4	....	19
	43	293	71.0	79	882	....	7.9	....	65
	45	152	85.5	87	646	....	4.6	....	30
	46	578	32.9	109	720	....	35.0	....	252
Mercer County	47	1,361	84.6	91	224	....	21.1	....	47
	48	313	92.3	105	898	....	6.5	....	59
	49	2,703	90.0	78	668	....	42.5	....	284
	50	176	94.3	109	467	....	14.5	....	68
	51	44	59.1	105	531	....	31.6	....	168

DISCUSSION OF DATA

For the determination of parasitization a total of 26,115 insects were reared from collections of infested twigs. In a few orchards, such as No. 34 in 1938 and No. 29 in 1939, the numbers reared are too small to permit accurate determination of the percentage parasitized for the orchard. In some orchards, as in Nos. 11 and 40 in 1937, the fruit load per tree departs widely from the normal. Since the tendency is toward large numbers of injured fruits and low percentages of injury with full crops and toward the converse with partial crops, such wide variations in crop size affect the measure of fruit infestation whether based on the numbers of injured fruits per tree or on the percentage of fruit injured. Despite the deficiencies and irregularities noted for some of the orchards, it is more desirable to include them in class groupings for the purpose of obtaining averages than to attempt arbitrarily to exclude some and to include others.

The values for fruit injury in table 1 include new injury and total injury. New injury is produced by the progeny of the moths that survive second-brood parasitization, and is therefore a better value for comparison with such parasitization than total injury, which includes older injury produced by larvae unaffected by second-brood parasitization. However, since determinations of new injury were not available for all orchards, total injury has been taken as the measure of fruit infestation in subsequent tables.

In table 2 the data for all the orchards from which comparable records were obtained are averaged for five classes, each covering a range of 20 percent of parasitization. In this arrangement there is no well-defined relation between the percentage of parasitization and the average fruit infestation. However, the class having 80- to 100-percent parasitization has the fewest injured fruits per tree and the lower percentage of injury. Only 3 observations in this class (No. 25 in 1938 and Nos. 49 and 50 in 1939) show more injured fruits per tree than the average of the lowest class (59.4), and only 1 (No. 49 in 1939) shows evidence of having, both in the number of injured fruits per tree and in percentage of injury, a greater degree of injury than the average of the classes of lower parasitization. On the other hand, in the 4 classes of less than 80-percent parasitization many observations show a fruit infestation lower than the average for the group having 80- to 100-percent parasitization. In the class of 60- to 79.9-percent parasitization 6 of the 13 observations are of this type, and in the successively lower classes the numbers are 6 out of 17, 2 out of 7, and 9 out of 24.

TABLE 2.—*Observations for second-brood parasitization in twigs and the total injury to peaches at harvesttime, averaged for 5 classes of parasitization*

Second-brood larvae parasitized, class range, in percent	Observations	Average injured fruits	Injured fruits per tree
	Number	*Percent*	*Number*
80–100.0	26	8.0	35.6
60–79.9	13	10.7	61.8
40–59.9	17	16.2	68.8
20–39.9	7	20.0	116.4
0–19.9	24	19.4	59.4

The probable explanation of this condition is that in orchards showing exceptionally high parasitization it is difficult for the fruit moth to increase, even in the absence of other controlling factors, whereas in orchards showing lower parasitization these other factors may frequently be of greater importance in determining the degree of fruit injury.

In 32 of the 51 orchards surveyed there are observations for 2 or 3 seasons. When the parasitization and the number of infested fruits are compared for each of the 32 orchards, the lower infestations are found to be associated with higher parasitization in the expected relation in only 12 orchards. When expressed in percentages, lower infestation is similarly associated with higher parasitization in only 14 orchards. It is therefore evident that in any orchard the occurrence of a parasitization higher or lower than that of the previous year is not necessarily followed by an equivalent change in the opposite direction in the amount of fruit infestation. Either the observed parasitization was unimportant in the control of the fruit moth, or its true importance was obscured by a high seasonal variability in the sum of the other factors determining moth abundance at harvesttime.

The comparison of data for individual orchards for successive years indicates that the relation between parasitization and fruit infestation may be obscured by important seasonal differences in the level of fruit moth populations, brought about by factors other than parasitism. In table 1 it is seen that in both 1938 and 1939 the levels of parasitization and of fruit infestation in New Jersey were quite different from those in the other districts surveyed. For these reasons a better basis for comparing parasitization with fruit infestation is obtained by restricting the comparisons within series to observations during the same year and for an area over which the sum of control agencies other than parasitism does not vary greatly.

TABLE 3.—*Injury to peaches by the oriental fruit moth in relation to the degree of parasitization of second-brood larvae as determined by orchards in Virginia, West Virginia, Maryland, and New Jersey in 1937, 1938, and 1939; orchards in each group arranged in order of decreasing parasitization*

1937 survey				1938 survey				1939 survey			
Orchard No.	Second-brood parasitization	Total injured fruits	Injured fruits per tree	Orchard No.	Second-brood parasitization	Total injured fruits	Injured fruits per tree	Orchard No.	Second-brood parasitization	Total injured fruits	Injured fruits per tree
Virginia and Maryland:	*Pct.*	*Pct.*	*No.*	Virginia, West Virginia, and Maryland:	*Pct.*	*Pct.*	*No.*	Virginia, West Virginia, and Maryland:	*Pct.*	*Pct.*	*No.*
7	82.2	3.3	22	32	98.6	0.6	6	27	84.8	1.1	3
31	69.4	6.0	8	6	96.4	2.0	12	25	81.5	1.7	20
33	63.8	3.3	47	4	95.6	4.0	24	23	81.0	2.3	25
1	56.7	8.3	60	28	93.7	1.6	3	32	80.6	.8	5
30	55.8	2.1	19	29	90.0	3.7	11	28	72.4	0	0
35	46.9	8.6	53	25	88.8	7.5	71	22	68.8	2.3	11
15	9.0	9.0	76	30	65.6	1.7	8	34	61.1	9.3	16
3	8.4	6.8	40	23	58.8	15.0	65	26	57.9	2.7	31
11	5.9	46.3	25	14	48.9	34.6	41	10	48.0	7.7	35
9	5.5	14.0	54	24	46.1	26.0	136	30	46.7	2.7	24
2	4.6	6.5	51	27	42.7	14.2	39	20	43.1	6.4	21
20	2.7	31.3	102	16	32.6	14.5	53	21	25.6	14.2	82
8	2.1	15.3	56	21	29.4	9.7	34	6	23.4	1.3	7
16	1.3	3.8	28	15	27.0	32.2	222	4	16.3	3.8	34
17	0	5.5	33	12	25.4	26.6	165	13	16.0	17.4	51
19	0	4.0	20	8	15.1	19.5	40	1	12.7	50.7	51
				20	13.9	12.2	17	5	11.6	4.8	28
				11	12.9	47.3	189	12	8.8	29.9	84
				9	5.2	42.0	40	3	8.8	18.4	138
								16	3.7	9.1	20
				New Jersey:				18	3.7	22.7	111
				42	88.9	3.0	13	11	2.9	39.2	122
				41	86.1	4.7	20				
				50	85.5	10.7	18	New Jersey:			
				47	84.8	26.2	20	50	94.3	14.5	68
				45	84.5	16.2	23	48	92.3	6.5	59
				48	83.1	5.2	30	39	90.0	12.7	53
				51	79.0	23.2	158	49	90.0	42.5	284
				44	77.4	17.5	40	38	85.8	3.9	26
				43	72.7	21.0	93	45	85.5	4.6	30
				38	63.2	3.7	8	47	84.6	21.1	47
				37	60.1	31.0	117	41	82.2	5.8	31
				49	59.6	17.5	69	43	71.0	7.9	65
				36	57.2	27.2	100	51	59.1	31.6	168
								42	58.4	15.4	19
								36	47.8	21.3	118
								37	46.9	34.5	171
								46	32.9	35.0	252

Such comparisons are shown in table 3, where the orchards in Virginia and Maryland have been grouped for the survey in 1937 and those in Virginia, West Virginia, and Maryland have been arranged in one series and those in New Jersey in another series for the surveys in each of the years 1938 and 1939.

From this arrangement it becomes evident that in each series the orchards with the higher rates of parasitization tend to have a lower fruit infestation than the orchards with the lower rates of parasitization. This is true for both values of fruit infestation. However, this relationship lacks uniformity when individual orchards are compared, since there are a number of orchards of low parasitization and low fruit infestation, such as Nos. 17 and 19 in 1937, and an occasional orchard of high parasitization and high fruit infestation, such as No. 49 in 1939.

It becomes desirable, therefore, to examine the data more critically to determine whether there is any significant relation between the parasitization observed and the measures of fruit infestation. For this purpose the orchards in each of the five series in table 3 were divided into two classes, one of high and the other of low parasitization. The point selected for separation was usually the midpoint of the observed range for the series.

If parasitization were not related to fruit infestation, the means of the classes of high parasitization would approximate the means of their paired classes of low parasitization, and the mean of lower fruit infestation would be likely to occur as often in one class as in the other. As shown in table 4, this situation does not exist. In each comparison between classes the mean fruit infestation of the class of high parasitization is lower than that of the corresponding class of low parasitization. This in itself is good evidence that there is an inverse relationship between parasitization and fruit infestation. The standard errors for the class means show not only that the differences between the classes are all in the same direction but also that they are large enough to be significant in 5 of the 10 class comparisons. When the significance of the class difference of the 5 series is calculated, it is indicated that the probability of these differences being significant is slightly more than 100 to 1 for the percentage of fruit infested and slightly less than 100 to 1 for the number of injured fruits per tree. It is concluded, therefore, that in this series of observations parasitization is inversely related to fruit infestation and the higher rates of parasitization produced correspondingly lower rates of fruit infestation.

TABLE 4.—*Averages of fruit injury for classes of high and low parasitization in the series of observations in orchards included in table 3*

Orchard series	Second-brood larvae parasitized	Observations	Total injured fruits	Injured fruits per tree
	Percent	*Number*	*Percent*	*Number*
Virginia and Maryland in 1937	46. 9–82. 2	6	5. 3±1. 1	35±9
	0 – 9. 0	10	14. 3±4. 4	49±8
Virginia, West Virginia, and Maryland:				
1938	58. 8–98. 6	8	4. 5±1. 7	25±10
	5. 2–48. 9	11	25. 3±3. 8	89±22
1939	43. 1–84. 8	11	3. 4± . 9	17± 3
	2. 9–25. 6	11	19. 2±4. 7	66±13
New Jersey:				
1938	77. 4–88. 9	8	13. 3±3. 1	40±17
	57. 2–72. 7	5	20. 1±4. 7	77±19
1939	71. 0–94. 3	9	13. 2±4. 1	74±27
	32. 9–59. 1	5	27. 6±3. 9	146±38

In table 4 the mean percentage of fruit infestation appears to be more closely correlated with parasitization than the mean number of injured fruits per tree. For the percentage value of the fruit injury in the Virginia-Maryland and the Virginia-West Virginia-Maryland series, the mean infestation in the orchards of high parasitization ranged from about one-third to about one-sixth of the mean infestation in those of lower parasitization. In the New Jersey series the mean range was smaller, a condition that may well have been due to the smaller range of observed parasitization in the series surveyed.

SUMMARY

During 1937, 1938, and 1939 a total of 51 orchards, located in 11 peach-growing districts in Virginia, West Virginia, Maryland, and New Jersey, were surveyed to determine whether there was a correlation between parasitization of twig-infesting larvae of the oriental fruit moth (*Grapholitha molesta* (Busck)) and subsequent infestation of the fruit. Comparisons were made (1) by arranging orchards in 5 classes according to percentage of parasitization, (2) by considering individual orchards, (3) by considering individual orchards over successive years, and (4) by grouping orchards by year within a given area.

It was found that, when the comparisons were limited to one season and a restricted district in which the conditions affecting fruit moth abundance, other than the parasitization measured, were reasonably uniform, high parasitization of second-brood twig-infesting larvae was followed by correspondingly low fruit infestation, with evidence that this relation was significant. This condition existed each year and throughout the area surveyed, but was more evident in 1938 and 1939 than in 1937, and in the orchards in Virginia, West Virginia, and Maryland than in those in New Jersey. When the observations were compared in any other manner, factors other than the observed parasitization, and apparently associated with the year and the locality, were sufficiently important to obscure the effect of second-brood parasitization in reducing fruit infestation.

These data support the findings of Daniel and his associates in Niagara County, N. Y., and of Yetter and Allen in Burlington County, N. J., that parasitization of twig-infesting larvae is an important factor in controlling infestation in ripe peaches.

THIAMINE IN CROWN GALL AS MEASURED WITH THE PHYCOMYCES ASSAY[1]

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INTRODUCTION

A better understanding of the growth factors that may exert an inciting influence on pathological cell growth seemed desirable for clarifying this diseased condition. Such factors can be studied with considerable facility by using crown gall, caused by *Phytomonas tumefaciens* (Smith and Town.) Bergey et al. This organism has appeared, in preliminary trials by McIntire, Riker, and Peterson (*24*),[2] to be relatively rich in thiamine, a substance known as a growth factor for many organisms, including higher plants. It seemed important, then, to determine whether there is a quantitative relation of thiamine to crown gall development.

The literature dealing with the role of thiamine in the growth processes of fungi and higher plants, respectively, has been reviewed by Schopfer (*45*) and Lilly (*19*); and by Bonner (*3*). However, certain papers that form the background of the present study are mentioned briefly.

Thiamine was demonstrated as a growth factor for higher plants, independently, by Kögl and Haagen-Smit (*16*), Bonner (*4*), and Robbins and Bartley (*32*). This work was done with excised embryos and roots in vitro. An increase in dry weight of some green plants as an effect of added thiamine has been reported by Bonner and Greene (*7*, *8*), Bonner and Bonner (*2*), and Bonner (*5*). Arnon (*1*) and Hamner (*13*) failed to confirm this beneficial effect on whole plants. Thiamine has been supposed (*29*) to function in respiration as a precursor of part of an enzyme system. No work has been found dealing with the effect of thiamine on pathological growths, such as crown gall. However, the requirement of thiamine by certain fungi makes possible its measurement.

The role of thiamine as a growth factor for *Phycomyces blakesleeanus* Bgf. was demonstrated, independently, by Schopfer (*36*) and Burgeff (*9*). The use of this fungus in a quantitative test for thiamine was proposed by Schopfer (*39*, *40*). Schopfer has done the pioneer work on the development of the *Phycomyces* assay, and the research of other investigators has been based largely upon his findings. It must be borne in mind that *Phycomyces blakesleeanus* is responsive not only to thiamine but also to the pyrimidine and thiazole fractions together (*20*, *34*, *42*, *44*, *47*), and to cocarboxylase (*20*, *48*), the pyrophosphoric acid ester of the vitamin. Hence, in estimating the thiamine content of plant tissues or extracts with the *Phycomyces*

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[2] Italic numbers in parentheses refer to Literature Cited, p. 108.

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method, the values obtained may be high because of the action of these other factors (cf. Schopfer, *37*, *41*, *45*, *46*).

Factors other than thiamine which may affect the vegetative growth of *Phycomyces* are of prime importance in assay work since the objective is that the dry weight of the mycelium produced shall be proportional to the available thiamine. The basic assay medium, then, should contain optimum concentrations of everything needed for the growth of *Phycomyces*, except thiamine. Accordingly thiamine, if limiting, might be determined quantitatively by the growth magnitudes of the fungus.

A synthetic medium containing 10 percent glucose, 0.1 percent asparagine, and inorganic salts was devised by Schopfer (*39*). He later (*42*) reduced the glucose content to 5 percent, then (*45*) to 3 percent. Bonner and Erickson (*6*), and McClary (*22*) used 10 percent glucose and 0.4 percent asparagine. Robbins (*28*) employed 5 percent glucose with 0.05 percent asparagine; also media with twice these concentrations. Robbins and Kavanaugh (*34*) used 10 percent glucose with 10 percent asparagine and later (*35*) reduced the asparagine to 1 percent. It is evident that there is lack of uniformity in the concentration of carbon and nitrogen sources used by various workers for the growth of *Phycomyces*. However, the relation of such food sources to growth and the desirability of standardization for assay purposes are recognized.

The interaction of constituents of the medium on the dry-weight yield of *Phycomyces* was studied by Schopfer (*38*). He found a pronounced increase in the dry weight of the mats with an increase in asparagine (0.05 to 4.0 percent), and a different optimum concentration of thiamine for each concentration of asparagine. The glucose content of the medium (1.0 to 15.0 percent) had no effect on the final dry weight of the mycelium when an excess of thiamine and 0.1 percent asparagine were present. Schopfer (*43*) obtained the best growth of *Phycomyces* on a medium of pH 4.0 or 5.0. There was a pronounced decrease in yield at pH 6.0.

The relation of various factors to the growth of *Phycomyces* was studied by Burkholder and McVeigh (*10*). With 4.0 and 8.0 gm. per liter of asparagine, and thiamine at 1×10^{-6}M, glucose was limiting up to quantities of 80.0 or 100.0 gm. per liter. At lower levels of asparagine content, 40.0 gm. per liter of glucose gave maximum growth. Definite increases in growth were obtained as the asparagine content was raised, if 20.0 gm. per liter or more glucose was present. They stated: "It appears that a medium containing 40 gm. per liter of glucose and 4 gm. per liter of asparagine permits good growth, although somewhat higher concentrations yield even more dry matter." Cultures were found to grow best at a pH of 3.5 to 4.6, and a temperature of 15° C.

In none of the reported experiments was material assayed under the varied environmental conditions to determine their effects on the assay results. All workers, of course, have not been primarily concerned with the use of *Phycomyces* as an assay organism. It does not seem necessarily true that conditions which give maximum growth in the presence of an excess of crystalline thiamine will be most favorable for assay use. With the assay, suboptimal concentrations of the vitamin must be used to get an increase in fungus dry weight corresponding to the increase in thiamine content of the medium. The unknown to be assayed must also be added to the basic medium in quantities which will yield mycelium in this linear range. Thiamine, crystalline or in the unknown material, must always be the limiting factor. In assay work, then, one is much less interested in the conditions inducing the most possible growth in a given volume of medium than in the slope of the growth curve. Thus it seemed necessary to determine the effects of various cultural conditions on the assay results, and then to adapt the assay method to the present problem.

The present studies, therefore, have included (1) adaptation of the thiamine assay to the present problem, (2) comparison of thiamine values of crown gall and healthy tissues, and the influence of various factors on these values, and (3) determination of the thiamine content of cultures of a virulent and an attenuated strain of *Phytomonas tumefaciens*. Such studies gave promise of clarifying the relationship of the plant growth factor thiamine to crown gall development.

An abstract of some of the present work has already been published (*15*).

MATERIALS AND METHODS

The Bonnie Best variety of tomato plants was used for crown gall inoculations, except where stated otherwise. The plants were grown individually in 4- or 6-inch pots in the greenhouse at approximately 24° C. They were inoculated when 6 to 8 inches tall by a needle puncture in each of three central internodes. A highly virulent strain (the A6 culture of Hendrickson, Baldwin, and Riker (*14*) of *Phytomonas tumefaciens* was used unless stated otherwise. Sterile punctures were made in control series of similar plants. Tissue samples for thiamine determinations included (1) galls from all of the three inoculation points, (2) stem segments of inoculated plants from the topmost gall to 1 inch below the terminal bud, (3) analogous segments from check plants, (4) mature, healthy leaves from inoculated plants, and (5) similar leaves from check plants. Any variations in this procedure are stated. Each sample included material from five or more plants. The temperature chambers described by Riker, Henry, and Duggar (*26*) were used in determining the effect of temperature on the thiamine content of inoculated and healthy plants.

The *Phycomyces* growth method was used for the determination of thiamine in the various tissues. Fresh or frozen-dried (*12*) tissue samples were used. The fresh samples were collected between 9 and 10 a. m., cut into about 5-mm. segments, and used immediately. Frozen-dried tissues were thoroughly ground with a mortar and pestle and used when needed. Extracts of the plant materials were employed since attempts to assay quantities of whole tissues gave very inconsistent results between duplicate samples, confirming results obtained by Schopfer (*41*).

Dry-weight determinations were made with duplicates of each sample assayed.

Two methods of extraction, with water and alcohol as the solvents, were used. Materials were water-extracted by autoclaving them for 15 minutes at 15 pounds' pressure in water 100 or more times their dry weight. The extract was then filtered or centrifuged, depending on the density of the original material, and the filtrate or supernatant liquid used for the assay. In alcohol extraction, a volume of 50-percent ethyl alcohol equivalent to the water in the other method was added to the material. The suspension was agitated continuously for 1 hour with an electric stirrer, filtered, and the material extracted again with a duplicate volume of fresh alcohol. After filtering the second suspension, the 2 filtrates were combined and concentrated under vacuum almost to dryness. The concentrate was brought to desired volume with distilled water. This solution was used for the assay. Both extraction methods were found suitable for the present studies since the calculated thiamine values obtained with water and alcohol extracts of samples of the same material were similar. Water extraction was employed in most of the work because of its simplicity.

The culture of *Phycomyces blakesleeanus* (+strain) used throughout these studies was secured from Prof. Leon H. Leonian. Stock cultures were maintained on the following medium: Thiamine (Merck's Betabion), 10.0 gamma; Bacto-dextrose (Difco), 5.0 gm.; amino acid mixture (*17*) (*d*-arginine, *d*-glutamic acid, *l*-aspartic acid, 2 parts each; *dl*-alanine, glycine, 1 part each), 0.5 gm.; ammonium nitrate, 0.5 gm.; potassium dihydrogen phosphate, 0.5 gm.; magnesium sulphate, 0.5 gm.; calcium carbonate, 1.0 gm.; Bacto-agar (Difco), 20.0 gm.; distilled water, 1,000.0 ml. Seventy-five-milliliter portions of this medium were added to 6-ounce bottles, autoclaved, slanted, and seeded with a spore suspension of *Phycomyces*.

The basic assay medium was prepared double strength, with magnesium sulphate, 0.5 gm.; potassium dihydrogen phosphate, 1.5 gm.; zinc, 0.2 p. p. m.; iron, 0.2 p. p. m.; manganese, 0.02 p. p. m.; Bacto-dextrose and nitrogen as indicated; distilled water to make 500.0 ml. The pH was adjusted to 5.5 with sodium hydroxide. To 50 ml. of this medium was added thiamine solution or plant extract, as the case might be, and the volume was then brought to 100 ml. with distilled water. Twenty-five-milliliter portions of this solution were added to each of four 250-ml. pyrex flasks, giving a quadruplicate series for each concentration. In a few experiments, as noted, duplicate series of 25-ml. portions in 250-ml. flasks were employed. The check series included concentrations of crystalline thiamine from 0 to 0.3 gamma per 25 ml. of medium. Plant extracts to be assayed were always included in two or three concentrations. The flasks were autoclaved at 15 pounds' pressure for 15 minutes. The spores for seeding the flasks were obtained from two stock cultures that had grown for 2 weeks at room temperature. With a hooked transfer needle the sporangia and sporangiophores were removed from the stock cultures and agitated in a tube containing 10 to 15 ml. of sterile distilled water. A drop of this spore suspension was used to seed each flask in a single series. Preliminary experiments indicated that, within a wide range, the amount of inoculum had no effect on the final yield of the fungus. The flasks were kept at 23° C. for selected incubation periods.

At the end of the incubation period, the mycelial mats were removed and thoroughly washed in water. This was done best by pouring distilled water down the side of the flasks so that the mycelium was turned over and over. When the flask was filled the mycelium was removed to an empty flask and again washed. The process was carried through three times, thus washing each mat in about three-quarter liter of distilled water. The mats were then dried to constant weight at 75° C., and weighed to the nearest milligram. The weight of the mat in each of the four flasks was determined separately, and the average weight per flasks was used in plotting the check curve and in calculating the thiamine content of an unknown. "Check curve" refers to the curve obtained by plotting the concentration of crystalline thiamine against the dry weight of *Phycomyces* produced. Thiamine values for the two or three concentrations of a given unknown were averaged to obtain the final value. Determination of the calculated thiamine value of an extracted unknown was as follows:

$$\frac{\frac{V}{v}B}{W} = \text{gamma of thiamine per gram dry weight of tissue extracted}$$

Where:

V = total volume of extract
v = volume of extract contained in 25 ml. of medium
B = gamma of thiamine per 25 ml. of medium which corresponds to the weight of mat of the unknown, as determined from the check curve
W = gram dry weight of the sample extracted

Example:

Where 2 gm. dry weight of tissue (W) is extracted with 200 ml. of water (V), 2.5 ml. of the extract (v) is included in 25 ml. of medium, and the dry weight of fungus mat produced corresponds to that produced by 0.1 gamma of crystalline thiamine (B) in 25 ml. of medium, then $\frac{\frac{200}{2.5}0.1}{2} = 4$, or gamma of thiamine per gram dry weight of tissue extracted.

ADAPTATION OF ASSAY METHOD

Various factors were studied before the assay was satisfactorily adapted to the present study. These included (1) some variations in the nitrogen and dextrose of the basic medium, (2) the growth period for *Phycomyces*, and (3) the validity of the assay. Alcohol extracts of the same lot of frozen-dried, tomato gall tissue were used unless stated otherwise. The flasks were incubated for 7 to 8 days, or as stated.

EFFECT OF VARIATIONS IN NITROGEN AND DEXTROSE

The source of nitrogen employed by most investigators for the growth of *Phycomyces* has been asparagine. Not a synthesized compound, it may vary between samples and contain impurities which necessitate repeated recrystallizations from water or alcohol. It is more expensive than synthetic glycine. Glycine (*18, 36, 43, 49*) and aspartic acid (*18*) have been shown to be practically as good as asparagine for the growth of *Phycomyces*. Leonian and Lilly (*18*) recommended glycine for assay media. Burkholder and McVeigh (*11*) concluded: "As a source of nitrogen, recrystallized asparagine may be preferable to glycine for thiamin assays with *Phycomyces*." However, no direct comparison of assay results obtained with the use of media containing each of these nitrogen sources was found. If glycine should prove feasible for assay work, it would thus be preferred over asparagine.

To determine the possibilities of using asparagine, aspartic acid, and glycine, each as the sole nitrogen source in the basic medium, they were employed at various concentrations. The *l*-asparagine (Pfanstiehl, C. P.) was recrystallized from distilled water three times to remove any thiamine present as an impurity. Glycine (Pfanstiehl, C. P.) and *l*-aspartic acid (Eastman) were not treated. The dextrose concentration was varied with asparagine and glycine. Thiamine values of gall extracts assayed on these different media are given in table 1. Only the values in a single experiment are directly comparable since a different extract was used for each. Water extracts were used in experiments 3 and 4.

TABLE 1.—*Some changes in the nitrogen and dextrose of the basic medium in relation to assay results*

Experiment No.	Nitrogen source	Nitrogen concentration per liter[1]	Dextrose concentration per liter	Thiamine per gram of dried tissue
		Gram	*Gram*	γ
1	Asparagine	2.0	25	5.7
			50	5.3
			100	5.1
		4.0	25	6.4
			50	6.0
			100	6.0
2	Aspartic acid	2.0	50	5.8
		6.0	50	5.5
3	Glycine	2.27	25	4.3
			50	4.3
			100	4.9
4	Glycine	1.14	50	5.7
		2.27	50	5.2
		4.54	50	6.6

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[1] On a total nitrogen basis, 2.0 gm. of asparagine is approximately equal to 4.0 gm. of aspartic acid, or 2.27 gm. of glycine.

Doubling the asparagine content of the medium gave a slightly higher assay value with each concentration of dextrose, as shown in experiment 1 of table 1. The difference at each dextrose concentration is of about the same magnitude as the widest variation between concentrations of the same extract, and thus is probably on the borderline of significance. Aspartic acid (experiment 2, table 1) gave no consistent increase in assay values with increase in concentration. Glycine (experiment 4, table 1) at 4.54 gm. per liter, however, gave a value 25 percent higher than at a concentration of 2.27 grams per liter. This corresponds with the results from the use of asparagine in experiment 1, though the increase in assay value is slightly greater. The slightly increased values at the higher nitrogen concentrations did not seem to justify a 100-percent increase in the nitrogen supply of the basic medium. The dry weight of the fungus mycelium in flasks with 0.2 to 0.3 gamma thiamine per 25 ml. was increased with the higher concentrations of asparagine and glycine. There was no such increase with aspartic acid. Thiamine was limiting in all these experiments; therefore, pronounced increases would hardly be expected (cf. Schopfer (*38*)).

The data in experiments 1 and 3 of table 1 show that increasing the dextrose concentration in the range employed, with either asparagine or glycine as the nitrogen source, has no pronounced effect on the assay results of gall tissues. The differences between the values in either experiment are within the range of widest variation between concentrations of the same extract. The lower values with glycine may be due to the different extract employed. Higher concentrations of dextrose increased the dry-weight yield of the fungus with asparagine in the presence of 0.2 and 0.3 gamma thiamine per 25 ml., and the highest concentration of plant extract (equivalent to 0.2 gamma thiamine per 25 ml.). There was no such increase with glycine. A concentration of 50 grams of dextrose per liter seemed in excess but not inhibitory, and so was used in all later assays.

It was thus demonstrated (table 1) that with thiamine limiting, as it must be for assay purposes, increases in the concentration of nitrogen and dextrose within the ranges employed did not affect the assay results.

Glycine, aspartic acid, and asparagine were each demonstrated (table 1) to be suitable nitrogen sources in the basic assay medium. To have an exact comparison of the three, it was necessary to assay samples of a single extract on media containing each as the sole nitrogen source. The data from such an experiment are recorded in table 2. The assay values with glycine and asparagine as nitrogen sources are almost identical. Aspartic acid gave an average value slightly higher than either of the others. Glycine, then, seemed as suitable a nitrogen source for the basic medium as asparagine, or aspartic acid, and was preferred because of its purity and relatively low cost. Therefore, glycine was used in all later assays, at 2.27 grams per liter.

TABLE 2.—*Thiamine values of a plant extract as affected by different nitrogen sources in the basic medium* [1]

Nitrogen source	Nitrogen concentration per liter [2]	Plant extract [3] per 25 ml. of medium	Thiamine in dried tissue	
			Per gram	Average
	Gram	*Milliliter*	γ	γ
Glycine	2.27	1.25	5.4	5.1
		2.5	4.7	
		3.75	5.1	
Aspartic acid	4.00	1.25	5.8	6.4
		2.5	6.9	
		3.75	6.4	
Asparagine	2.00	1.25	4.8	5.3
		2.5	5.4	
		3.75	5.7	

[1] Dextrose at 50 grams per liter was employed with each.
[2] Concentrations are approximately equal on a total nitrogen basis.
[3] Extract of 2.5 grams of gall tissue contained in 250 milliliter of water.

EFFECT OF GROWTH PERIOD

The shortest time that allows maximum growth of *Phycomyces*, at all concentrations of thiamine and plant extract employed, seemed desirable for assay work. Schopfer (*38*, *43*) found that maximum growth occurred in 7 to 9 days in 20 to 25 ml. of medium at 23° C. Some reduction in fungus weight was usually evident after 14 days, with 0.4 to 64.0 gamma of thiamine per liter. Burkholder and McVeigh (*10*) stated: "No loss in weight of the fungus occurred at 25° [C.] even at the end of thirteen days." Their initial concentration of thiamine was 10^{-7} molar.

Two experiments were performed to determine the incubation period most suitable for assay. In the first experiment, duplicate flasks of each concentration of the check thiamine series and of the plant extract series were removed after 5, 6, 7, and 8 days' growth. In the second experiment duplicates were removed at 6-, 7-, 8-, and 9-day intervals. The data of the second experiment, plus the fifth-day data of the first, are recorded in table 3 as the average dry weight of mycelium per flask. The results at 6, 7, and 8 days were similar in the two experiments.

TABLE 3.—*Growth quantities of Phycomyces as related to time period in media with different concentrations of crystalline thiamine and plant extract, alone and combined*

Thiamine in 25 ml. of medium (γ)	Extract [1] in 25 ml. of medium	Average dry weight of fungus per flask at given time (days) after seeding				
		5	6	7	8	9
	Milliliter	*Milligram*	*Milligram*	*Milligram*	*Milligram*	*Milligram*
0.0	0	0	1	0	0	0
.05	0	20	23	26	19	20
.1	0	25	38	42	39	37
.2	0	23	72	77	80	77
.3	0	32	95	110	99	105
.75	0	41	155	160	147	157
1.5	0	45	159	167	161	170
2.25	0	38	161	167	164	172
.0	5	39	54	-----	58	56
.0	10	78	94	101	99	101
1.5	5	117	174	175	177	177
1.5	10	-----	199	190	184	197

[1] 250 milliliter water extract of 1 gram of cold-dried, tomato gall tissue.

The maximum growth of *Phycomyces* in these trials was reached 7 days after inoculation. There was a very rapid increase in growth of the fungus between the fifth and sixth day after seeding. No decrease in fungus weight was evident at 9 days after seeding, except with the lower concentrations (0.05 and 0.1 gamma per 25 ml.) of thiamine. Since assays had been terminated after 8 days' growth in most of these preliminary studies, it seemed desirable to compare the thiamine values obtained at 7 days with those obtained at 8 days.

In several assays, duplicate flasks were removed at 7 and at 8 days and the thiamine values of extracted tissues determined at each of these intervals. The comparative thiamine values (gamma per gram) of five extracts, each determined at 7 and 8 days, respectively, were (1) 5.3 and 4.9, (2) 11.4 and 11.9, (3) 3.1 and 2.9, (4) 8.2 and 7.9, and (5) 12.2 and 12.2. The extracts were of different plant tissues, hence the variation in magnitude of thiamine content. These data show that the difference between assay results determined at 7 and at 8 days is negligible. All later assays were terminated at 7 days because of the convenience of the 1-week interval.

VALIDITY OF ASSAY

The validity of the *Phycomyces* assay is demonstrated to some extent by the data in table 2. Three concentrations of the same extract were used with all of the 3 media. These concentrations contained thiamine within the quantitative range (0.05 to 0.3 gamma per 25 ml.) of the check curve. If thiamine were the only factor in the extract which limited the growth of *Phycomyces*, the 3 extract concentrations in a single medium should give identical calculated thiamine values. However, the values were not always identical; but neither did they show a consistent increase toward the highest or lowest concentration of the extract. Later experiments showed both more and less variation than was evident in table 2. It seemed unlikely that factors other than thiamine in the extract would influence the growth of *Phycomyces* to exactly the same degree as this vitamin. Hence, the fact that the thiamine values, determined from each of 3 concentrations of the same extract, were similar indicated that the assay was valid for the extracts used. Part of the deviation was probably due to manual error. Further evidence indicating the presence of interfering factors was obtained when combinations of crystalline thiamine and plant extracts were used.

In a medium containing an optimum concentration of crystalline thiamine (i. e., 1.5 gamma per 25 ml., under the conditions of this experiment), plus plant extract, the vegetative growth of *Phycomyces* at 5 days after seeding was pronouncedly increased over that in the same medium containing the thiamine alone (table 3). The fungus mat produced by the combination of thiamine and plant extract exceeded the combined weights produced by the two when employed separately. The higher concentration of plant extract, alone, exhibited a similar but not so pronounced stimulation of early vegetative growth. The cause of this stimulation may have been similar to the "factor Z" reported by Robbins (*30, 31*) and Robbins and Hamner (*33*). Regardless of the cause, it seemed doubtful if the stimulation of early vegetative growth affected the assay results since the effect was obscured by the seventh day in media containing extracts which gave values within the quantitative thiamine range (table 3). Robbins (*31*) stated: "Whether factor Z affects the dry weight of the mature mycelium of *Phycomyces* when thiamine is present in limited quantity is uncertain." Where thiamine was not limiting, however, the stimulating effect of extracts on maximum growth continued through 9 days (table 3).

The combination of plant extract with an optimum concentration of crystalline thiamine gave a greater yield of mycelium at 6, 7, 8, and 9 days each than did the thiamine alone (extracts plus thiamine, table 3). The increase was greater with the higher concentration of plant extract. This experiment has been repeated with the same results. Since thiamine was not limiting, there must have been another factor in the extract which brought about the increased growth. Perhaps this was the same factor that stimulated early vegetative growth. However, this factor should not influence the assay unless it is evident when combinations of thiamine and plant extract are employed within the quantitative range.

Assuming that the growth of *Phycomyces* on the basic assay medium is determined by the thiamine added, it would be expected that crystalline thiamine and plant extract in combination would produce an effect equivalent to their combined thiamine values when supplied within the range of quantitative sensitivity. This idea was tested in several experiments. The results of 4 of these trials are presented in table 4. In the first trial there was an exact additive effect. Crystalline thiamine and plant extract, in combination, produced a growth response identical with that of a crystalline thiamine quantity equivalent to the sum of their thiamine values. In the other 3 trials, however, the actual thiamine values of the combinations were less than expected on the basis of a purely additive effect. In no case was there evidence of the "other factor" discussed in preceding paragraphs. The cause of these lower-than-expected values, though unknown, may be an inhibiting or toxic factor, or factors, in the extract (*11*).

TABLE 4.—*Effect on the growth of Phycomyces of combinations of crystalline thiamine and plant extracts within the range of quantitative response*

Trial No.	Source of extract	Thiamine value [1] of extract in medium (per flask)	Crystalline thiamine in medium (per flask)	Expected values [2]		Actual values [3]	
				Total thiamine in medium (per flask)	Dry weight of fungus (per flask)	Total thiamine in medium (per flask)	Dry weight of fungus (per flask)
		γ	γ	γ	*Milli-grams*	γ	*Milli-grams*
1	Fresh tomato galls	0.07	0.1	0.17	63	0.17	63
2	do	.112	.1	.212	74	.19	69
3	do	.045	.1	.145	58	.095	42
4	Dry tomato galls	.103	.1	.203	88	.168	73

[1] The concentration of thiamine on the check curve, which corresponds to the same dry weight of fungu as that produced by the extract.

[2] On the assumption that thiamine plus plant extract would give a growth effect equivalent to that produced by a quantity of thiamine equal to their combined values. For example, in trial 1 the expected thiamine value, 0.17 gamma, is the sum of the thiamine value of the extract (see footnote 1), 0.07 gamma, plus the crystalline thiamine added, 0.1 gamma. The expected growth value, 63 mg., is the weight of fungus produced per flask with 0.17 gamma of thiamine per 25 ml., as determined from the check curve.

[3] Actual thiamine value is the thiamine concentration corresponding to the same dry weight of fungus on the check curve.

Such inhibiting factors were occasionally evident with the highest concentrations of the extract series in later experiments. The degree of inhibition varied greatly between experiments. Conditions which determined the presence or absence of these factors are unknown. In 6 of the 65 experiments made during this study, toxic materials in the tomato extracts prevented sufficient growth of *Phycomyces* for the collection of significant data.

The validity of the *Phycomyces* assay was checked against 2 other procedures. Identical results were secured with this and a chemical method based on azo dye formation in assaying water extracts of rye germ.[3] However, of the 2 procedures, the *Phycomyces* assay was much more applicable to measuring low concentrations of thiamine such as are found in rye middlings. Likewise the *Phycomyces* assay and the "chick" assay (*23*) gave comparable results, as discussed later.

ASSAY METHOD AS ADAPTED

As a result of these preliminary experiments, the basic medium for later assays contained 50.0 gm. per liter of Bacto-dextrose, 2.27 gm. per liter of glycine, and the mineral salts as listed earlier. The cultures were incubated for 7 days at 23° C. Water extracts of fresh plant tissues were used, unless stated otherwise. Other phases of the method were unchanged.

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[3] IHDE, A. J. STUDIES ON SOME OF THE MINOR COMPONENTS OF THE RYE GERM. 1941. [Ph. D. thesis. Copy on file in the University of Wisconsin library, Madison, Wis.]

As representative of data secured in later assays, results with crystalline thiamine and two plant extracts of the December 9, 1940, assay are given in table 5. The calculated thiamine values of the two concentrations of each extract were almost identical—illustrating again the dependability of the assay. Extract of the control stems showed the most variation, with a difference of 0.6 gamma between the two calculated thiamine values. The widest variations between replicate flasks were less than 10 percent in most cases. These larger variations were probably due to manual error. The data from the crystalline thiamine series in table 5 may be compared with those in figure 1.

TABLE 5.—*Representative data from the Phycomyces assay of December 9, 1940*

Materials included per 25 ml. of basic medium	Dry weight of fungus per flask: Replicates				Dry weight of fungus per flask: Average	Calculated thiamine values [1]: From each extract concentration	Calculated thiamine values [1]: Average
	Milligrams	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	γ	γ
Crystalline thiamine (γ):							
0	3	3	1	2	2		
0.05	27	28	26	26	27		
0.1	45	47	45	45	46		
0.2	78	75	79	80	78		
0.3	111	106	113	103	108		
Crown gall, extract (milliliters): [2]							
5	39	44	38	40	40	7.7	7.7
10	64	65	73	67	67	7.6	
Control-stem extract (milliliters): [3]							
5	26	30	29	26	28	2.4	2.7
10	54	56	64	54	57	3.0	

[1] Per gram dry weight of tissue extracted.

[2] A 100-ml. water extract of 2.5 gm. of fresh tomato gall tissue (8.6 percent, dry weight).

[3] A 100-ml. water extract of 5.0 gm. of fresh, control, tomato stem tissue (9.4 percent, dry weight).

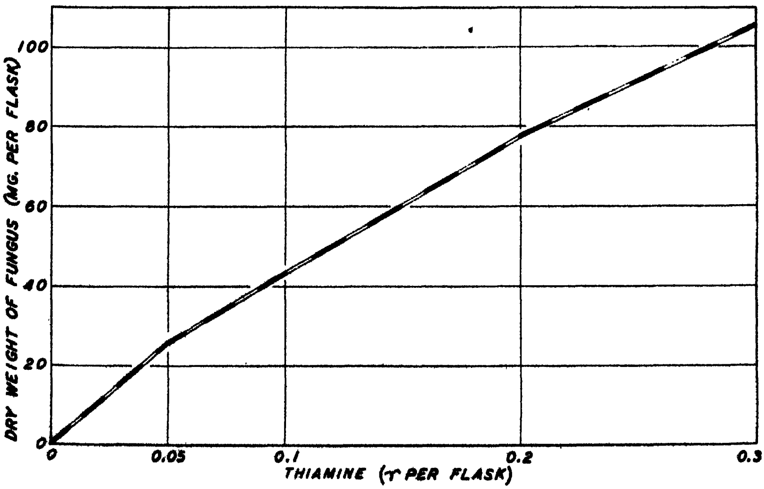

FIGURE 1.—The relation of the growth of *Phycomyces* to the concentration of crystalline thiamine in the glycine medium. Each 250-ml. flask contained 25 ml. of medium.

The relation of crystalline thiamine concentration to the growth of *Phycomyces* is graphically recorded in figure 1. Points on the curve were established from the average data of all (*31*) check curves in which these particular concentrations of thiamine were used. The data were obtained during the period from August 1940 to April 1941. Individual curves varied only slightly from the average. The nearly linear relation of the fungus weight to thiamine concentration in the basic medium is evident from these data. Thus, the check curves met experimental requirements.

EXPERIMENTAL RESULTS

THIAMINE IN CROWN GALL AND HEALTHY TISSUES

The thiamine concentration in gall and stem tissues was determined in numerous experiments with greenhouse tomatoes, three experiments with greenhouse sunflowers, one with field marigolds, and two with field tomatoes. Typical data from greenhouse tomato plants are given in table 5. In this case the concentration of thiamine in gall tissue was nearly three times that in the control stems. Most of the data are omitted because of their similarity to those presented. Preliminary experiments thus showed the thiamine content of crown gall tissue to be consistently higher than that of healthy-stem tissue from analogous regions of check plants.

The consistently high thiamine concentration in gall tissue led to further experiments aimed at clarifying the relation of thiamine to gall development. If thiamine were a causal factor in crown gall development, its concentration in the galls might be expected to increase or decrease as they increased in size. A change in thiamine concentration in the galls might also be associated with a concentration change in other parts of the inoculated plants. To determine these relationships, the thiamine content of the leaves, stems, and galls of diseased plants was determined at intervals up to 5 weeks after inoculation. Leaves and stems of healthy plants were assayed for comparison. Six series of tomato plants, each including diseased and healthy individuals, were used. Determinations were made at such intervals that each value in the composite results, given in table 6, included the averaged data from two or three series of plants.

TABLE 6.—*Thiamine in parts of diseased and healthy tomato plants at progressive intervals after inoculation*

Weeks after inoculation	Thiamine per gram dry weight in—				
	Inoculated plants			Check plants	
	Galls	Leaves	Stems	Leaves	Punctured stems
	γ	γ	γ	γ	γ
1	[1] 7.0	7.5	3.7	7.1	4.1
2	[1] 5.1	6.8	4.1	6.0	5.2
3	7.9	4.2	2.2	5.0	2.9
4	8.8	6.5	4.1	7.3	3.1
5	9.3	5.4	4.0	7.7	4.1

[1] That section of the stem containing the inoculation point was used here since the galls were not sufficiently developed to be separated from the stem tissue.

It appears from the data in table 6 that, at least through the first 5 weeks of development, the gall tissue was higher in thiamine content than either leaves or stems. At 1 and 2 weeks after inoculation the leaves of both inoculated and check plants gave higher values than the "gall"; but the fact must be considered that there was no gall, as distinct from stem tissue, during the first 2 weeks and hence the "gall" tissue assayed contained stem material (fig. 2). However, thiamine

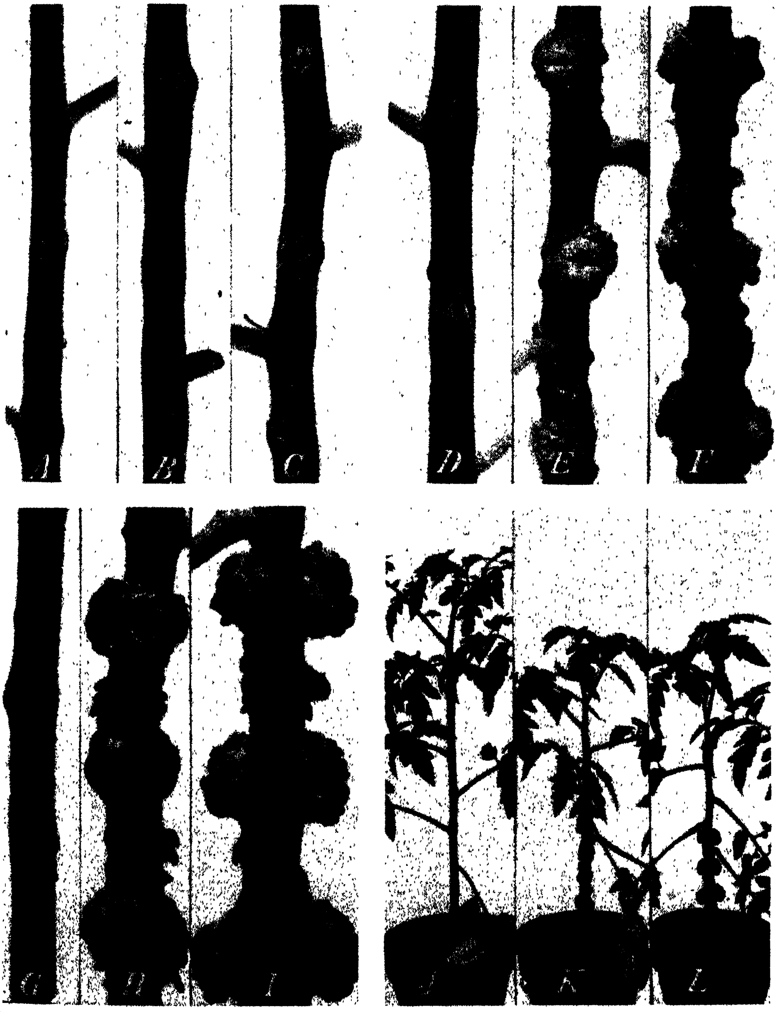

Figure 2.—Stages in the development of crown gall on tomato: *A*, *B*, and *C*, 10 days after inoculation; *D*, *E*, and *F*, 20 days after inoculation; *G*, *H*, and *I*, 30 days after inoculation; *J*, *K*, and *L*, whole plants from which the segments *G*, *H*, and *I*, respectively, were taken. *A*, *D*, *G*, and *J*, control plants; all others, inoculated. Inoculated plants (*K* and *L*) are dwarfed in comparison with control plant (*J*). *A–I* approximately natural size; *J–L* about one-sixth natural size.

was evidently being rapidly accumulated at the inoculation point since the value at 1 week was considerably higher than in stem tissue of either inoculated or control plants. The leaves contained more thiamine than the stems. Values for stem tissues in inoculated and check plants were similar. Values for leaf tissues were similar up to 3 weeks after inoculation. Beyond 3 weeks, however, the leaves of inoculated plants were lower in thiamine concentration than the leaves of control plants of similar ages. The thiamine concentrations in the leaves and stems of control plants and the stems of inoculated plants did not change significantly with an increase in age and size. The galls increased slightly in thiamine concentration from 3 to 5 weeks after inoculation, during the period when true gall tissue was assayed. Though the increase in concentration was slight, the increase in content was much greater because of the increasing size of the galls. There is thus the possibility that the galls were aided in enlarging by the transport of excessive quantities of thiamine from the leaves, hence the decreasing concentration of thiamine in the leaves of inoculated plants as compared with those of control plants.

TOTAL THIAMINE IN INOCULATED AND CONTROL PLANTS

As has been noted in earlier studies, inoculated plants are usually shorter than controls (fig. 2). This stunting becomes more noticeable with increased time after inoculation, correspondingly as the galls increase in size. Thus it seemed that the galls might be depleting other parts of the inoculated plant of necessary factors for growth, e. g., thiamine. The data in table 6, discussed in the preceding section, indicated that on a unit dry-weight basis thiamine was possibly being depleted in the leaves of inoculated plants.

To supplement these data, it seemed necessary to assay whole plants from inoculated and control series and compare their respective thiamine values. The above-ground portions of plants were assayed for thiamine at 4 to 5 weeks after inoculation. From three to five whole plants were used in the preparation of each extract. The thiamine values are thus representative of the entire aerial portions of the plants, not of any specific tissue. The average thiamine value for inoculated plants from three experiments was 5.2 gamma per gram dry weight, and for control plants from identical experiments, 5.7 gamma. Inoculated plants averaged 2.8 gamma dry weight each, making the thiamine content 14.6 gamma per plant. Control plants averaged 3.4 gm. each, giving a thiamine content of 19.4 gamma per plant. The dry weight of both types of plants was roughly 10 percent of the fresh weight. Thus, control plants contained more thiamine though most of the difference was accounted for by the greater weight of the plants.

The similarity of the thiamine values per gram of dry weight for the two types of plants was also shown indirectly as follows: The total weight of the control plants consisted of about 60 percent leaf and 40 percent stem tissues; inoculated plants consisted of about 45 percent leaf, 40 percent stem, and 15 percent gall tissues. Thus the relative amounts of stem tissues in the two types of plants were equal; inoculated stems were shorter but much thicker in the region of the galls. Leaves and galls combined were the same percent of the total weight of inoculated plants as were leaves alone of the control plants.

The gall tissue, then, seemed to have been formed almost entirely at the expense of the leaves. When the thiamine concentrations of the two types of plants was computed from the assay values of the individual parts (galls, leaves, stems) and their respective percentages of the whole plants, given above, the thiamine concentration per gram dry weight of inoculated plants was 5.7 gamma, and of control plants, 5.9 gamma. These theoretical values compare well with the actual ones of 5.2 gamma per gram for inoculated plants, and 5.7 gamma per gram for control plants.

DISTRIBUTION OF THIAMINE IN INOCULATED PLANTS

Concentration of thiamine, as a growth factor, might be expected to be different in meristematic regions of the plant from that in regions that have reached maturity. Crown gall consists largely of tissue of a meristematic nature. Inoculated plants, then, have two aerial meristematic regions: (1) The apical tip, and (2) the region of inoculation. The distribution of thiamine in inoculated plants was studied with particular concern for correlation between the concentrations of this vitamin in the two areas of meristematic activity.

The concentration of thiamine in the following tissues of inoculated plants was determined about 4 weeks after inoculation: (1) Galls, (2) leaves, and (3) sections of the stems above the topmost galls. Stem sections were divided into 1.5-inch segments. The segments from the top (including the terminal bud), central, and bottom areas of the sections were assayed separately, giving three thiamine values for stem tissues. The thiamine concentration per gram dry weight of tissues from four replicates of the above experiment were averaged for the following values: Galls, 8.1 gamma; leaves, 6.8 gamma; top stem segments, 9.6 gamma; central stem segments, 5.8 gamma; and bottom stem segments, 5.6 gamma. The thiamine concentration in the stems thus showed a definite gradient which fell rapidly below the meristematic region.[4] Galls were higher in thiamine concentration than leaves, and both were higher than the mature stem areas but considerably lower than the stem tips. However, the thiamine concentration of the galls did approach that of the stem tips. This similarity may be due in part to the meristematic nature of gall tissue.

THIAMINE IN PLANTS GROWN ABOVE AND BELOW THE MAXIMUM TEMPERATURE FOR GALL FORMATION

It has been shown by Riker (*25*) that crown galls develop well on tomato at 28° C. but fail to develop on this host at 32°. If thiamine were the limiting factor for gall formation, it might be expected that plants grown at 32° would show a deficiency in the vitamin to account for the failure of gall development. A series of plants was inoculated and one-half of them transferred to each of two chambers, regulated to 28° and 32° C., respectively. After 5 weeks the plants were removed and stems, leaves, and galls assayed for their thiamine content. Galls had developed only at the lower temperature. The thiamine values per gram of dry weight for the 28° galls, leaves, and stems, respectively, were 8.4, 7.0, and 4.6 gamma; for the 32° leaves and stems, respectively, 6.9 and 5.1 gamma. A similarly treated series of plants inoculated with a partly attenuated bacterial culture gave corresponding results. Since the higher temperature evidently had no effect on the concentrations in the host plants, thiamine deficiency could not have prevented gall development.

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[4] After completion of the present manuscript, the following publication appeared reporting a similar gradient in tomato stems: BONNER, J. TRANSPORT OF THIAMIN IN THE TOMATO PLANT. Amer. Jour. Bot. 29: 136–142. 1942.

THIAMINE IN GALLS FROM VIRULENT AND PARTLY ATTENUATED BACTERIAL CULTURES

A partly attenuated culture, the A6–6 strain of Hendrickson, Baldwin, and Riker (*14*), of *Phytomonas tumefaciens* erratically produces small galls, a few millimeters in diameter, on tomato. If thiamine were limiting the growth of the A6–6 galls, it might be expected that the concentration of this vitamin would be considerably less in them than in the galls produced by the virulent culture (A6).

A series of plants was inoculated with each of the cultures. Uninoculated, but punctured, plants served as controls. Thiamine assays were made on the various plant parts 8 weeks after inoculation. The thiamine concentration per gram of dry weight of the A6–6 galls was 7.8 gamma, and of the A6 galls, 8.8 gamma. On the basis of thiamine content per gall, the values for A6 galls were much higher because of their relatively greater size. The thiamine concentrations in stems and leaves, respectively, were similar in the two types of inoculated plants, and in control plants.

When tomato stems are inoculated with the A6 strain and, a few millimeters below, with the A6–6 strain, the latter produces galls similar in size to the A6 galls above them (*21*). This effect may be caused by some growth factor which diffuses from the region of the A6 inoculation and accelerates the A6–6 gall development. Galls from two such series of plants were assayed for thiamine 6 weeks after inoculation. The average thiamine value per gram dry weight of the A6 galls was 8.5 gamma; of the A6–6 galls, 8.8 gamma. The thiamine concentration in these A6–6 galls, equal in size to the A6 galls, was only 1 gamma per gram greater than in the A6–6 galls which were alone on the plant and showed practically no development. Thus, the thiamine concentration in galls from the partly attenuated culture was increased but little when they were stimulated to greater growth.

To determine whether thiamine had any of the stimulating properties of the virulent bacterial culture when applied above inoculations of the A6–6 strain, thiamine in lanolin was applied to the decapitated tips of 45 plants inoculated with the attenuated bacteria. One-third of the series was treated with plain lanolin, one-third with 0.3 percent thiamine in lanolin, and one-third with 3.0 percent thiamine in lanolin. After 1 week, the plants were decapitated for the second time and fresh lanolin mixtures applied. The experiment was terminated 4 weeks after the first treatment. There were no observed differences between the thiamine-treated and the nontreated plants in their reaction to A6–6 inoculations. Though stems of the plants, minus the lanolin-covered tips, were assayed for thiamine at weekly intervals, the results were so erratic that it could not be certain how much of the added vitamin reached the inoculation points. Thus, from this evidence, thiamine alone did not seem to possess the stimulating properties of the virulent culture.

COMPARISON OF THIAMINE CONTENT OF VIRULENT AND PARTLY ATTENUATED BACTERIA

The virulent strain (A6) of *Phytomonas tumefaciens* had been shown (*24*) to be relatively rich in thiamine. It seemed desirable, therefore, to compare this strain and the partly attenuated one (A6–6) in regard to their thiamine concentrations. If the A6–6 strain synthesized less thiamine in culture, it might act likewise when introduced into a host plant and so influence the host reaction.

Several cultures of the two strains were prepared for thiamine assay.[5] The bacteria were grown in a synthetic medium (*23*). Two hundred-milliliter portions of the medium were added to 1-liter serum jars and autoclaved. The jars were seeded with the desired bacterial culture and incubated for 5 to 6 days at 28° C. The cultures were aerated with the aid of an automatic shaker for the entire incubation period. At the end of this period the cultures were centrifuged and the cells assayed for thiamine.

The average thiamine value per gram dry weight of cells from seven cultures of A6 was 17.5 gamma, and from five cultures of A6–6, 22.1 gamma. The value for A6 cells is higher than that (12.0 gamma per gram) determined by the "chick assay" and reported by McIntire, Riker, and Peterson (*24*). More rapid growth in the present improved medium and the pyrimidine and thiazole fractions of thiamine, possibly present in the cells, may account for the higher value obtained with *Phycomyces*.

Attempts to assay the cellfree medium failed because of the presence of a substance, or substances, inhibitory to the growth of *Phycomyces*.

DISCUSSION

The lack of uniformity in the conditions under which the *Phycomyces* assay had been used by various workers necessitated some studies on the method preceding its adaptation to the present problem. Though most workers have used asparagine as the source of nitrogen in the assay medium, it has the disadvantage of not being practically available in synthetic form. It must therefore be treated to remove the thiamine which may be present as an impurity. The amount of thiamine and other impurities may also vary between different lots of asparagine. It was in an attempt to overcome these disadvantages that glycine was tried as the nitrogen source. Glycine had previously been reported as suitable for the growth of *Phycomyces* (*18*, *36*, *43*, *49*) and suggested for use in the assay medium (*18*). The present studies demonstrated the applicability of this compound to assay use, and it was preferred to asparagine (1) because of the more nearly linear relation of the weight of the fungus mat produced to the thiamine concentration in the medium (i. e., a more nearly straight "check curve"), (2) because of its relative purity, and (3) because of its relatively low cost. A basic medium containing 2.27 gm. per liter of glycine, 50.0 gm. per liter of Bacto-dextrose, and inorganic salts proved quite satisfactory. The medium is less concentrated than that used by many workers. However, with thiamine limiting, increasing the nitrogen supply gave only slightly higher assay values, and increasing the dextrose supply had no apparent effect on the values. Therefore, the more concentrated media, which provide maximum growth with an excess of thiamine, need not be the best for assay use where thiamine must always be limiting.

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[5] Dr. F. C. McIntire gave valuable assistance in the bacterial culture work.

A growth period of 7 days for *Phycomyces* was the nearest optimum with all concentrations of thiamine (0.05 to 0.3 gamma per 25 ml.) and plant extracts under the environmental conditions used. There was a very rapid increase in growth between 5 and 6 days after seeding. After 9 days' growth there was no decrease in fungus weight except in cultures with very limited supplies (0.05 and 0.1 gamma per 25 ml.) of thiamine. Schopfer (*38*, *43*), and Burkholder and McVeigh (*10*) reported no loss in fungus weight for even longer growth periods when an excess of thiamine was present.

The *Phycomyces* assay seemed to be valid since (1) different concentrations, or quantities, of an extract gave similar calculated thiamine values; (2) crystalline thiamine and plant extract combined exerted a growth influence equal to their combined thiamine values, except where the inhibitive action of the extract seemed evident; (3) "other factors" in the plant extracts which stimulate growth of *Phycomyces*, even with an optimum concentration of thiamine present, were not evident at 7 days in media containing the extracts within the quantitative thiamine range; and (4) thiamine values of bacterial cells compared well with those obtained with the "chick assay" and reported by McIntire, Riker, and Peterson (*24*).

For determinations of thiamine in plant tissues, the *Phycomyces* assay is quite suitable since minute quantities may be detected in relatively crude tissue extracts. The method as used was sensitive to differences of 0.01 gamma of crystalline thiamine per 25 ml. The sensitivity to changes in the plant extracts was less, but still sufficiently quantitative for comparative purposes. Factors inhibiting the growth of *Phycomyces*, as sometimes encountered with tomato extracts, caused the most serious difficulty.

The present studies have shown that thiamine concentration in crown gall tissues was consistently higher than in mature leaf or stem tissues of the same plant or similar tissues from uninoculated plants. Leaves contained more thiamine per unit of dry weight than mature stems, but usually less than galls. Thiamine was present at the inoculation points in almost maximum concentration within 1 week after the bacteria (*Phytomonas tumefaciens*) were introduced. Hence there was not much increase in thiamine concentration during the period of rapid increase in gall size.

As the galls increased in size, thiamine concentration in the leaves of inoculated plants tended to decrease. This, in addition to the much lower percentage of leaf tissue in inoculated plants than in control plants, suggested that the galls were depleting the leaves of a normal complement of thiamine, thus contributing to the dwarfing of inoculated plants.

Two reasons are offered for the relatively high thiamine concentration in the gall tissues. First, the bacterial cells are rich in the vitamin, as shown by McIntire, Riker, and Peterson (*24*) and confirmed in the present studies. It is noteworthy that the number of bacteria reaches the maximum during the first week (*27*) when the thiamine concentration is almost at the maximum. Second, the gall tissue is meristematic in nature and, as was shown in the case of stem tips, this type of tissue is considerably higher in thiamine concentration than mature or nearly mature tissues.

Thus, the higher thiamine concentration in crown galls than in mature, normal tissues from adjacent regions of the same plant or similar regions of healthy plants, is probably due, in the early stages of gall development, more to the presence of crown gall bacteria than to the meristematic nature of gall tissue. With progressive development, however, the relative importance of these causes is possibly reversed.

Other lines of evidence, discussed under their individual headings, failed to indicate that thiamine played a causal role in crown gall initiation or development. These included comparisons of the thiamine concentration in inoculated and control plants, the effect of temperatures above and below the maximum for gall formation on the thiamine concentration in the host plant, and comparisons of the thiamine concentrations in virulent and partially attenuated bacterial cells and galls produced by these cells.

It appears, therefore, that thiamine alone does not have a causal role in crown gall initiation or development beyond that of any necessary food or growth factor transported to, or produced in, that area of meristematic activity.

SUMMARY

Some relations of thiamine to crown gall development have been studied. The *Phycomyces* assay was used for all thiamine determinations.

Glycine, aspartic acid, and asparagine were compared as nitrogen sources in the basic medium for *Phycomyces*. Glycine was selected as the most suitable source because of its relative purity, its relatively low cost, and the more nearly linear relation of thiamine concentration in the new glycine medium to the dry weight of *Phycomyces*.

A basic assay medium containing 2.27 gm. per liter of glycine and 50.0 gm. per liter of dextrose was most satisfactory.

At 23° C. an incubation period of 7 days gave optimum growth at all concentrations of crystalline thiamine and plant extract employed.

Thiamine concentration in crown gall tissue was consistently higher than in mature, or nearly mature, stem tissues from inoculated or check plants. This relationship was found with greenhouse and field tomatoes, greenhouse sunflowers, and field marigolds as host plants.

Thiamine accumulated in almost maximum concentration at inoculation points within 1 week after treatment, and before macroscopic galls were evident.

The thiamine concentration in galls remained fairly constant from 3 to 5 weeks after inoculation, during the period of rapid increase in gall size, and approached that in the growing tip of the host plant.

Mature leaves contained more thiamine per unit of dry weight than mature stems, but usually less than galls.

The content of thiamine per unit dry weight of the whole aerial portions of inoculated tomato plants was similar to that of comparable portions of control plants. Control plants contained more thiamine, however, because they were larger.

Temperatures above (32° C.) and below (28° C.) the maximum for gall formation on tomato had no apparent effect on the thiamine concentration in various tissues of inoculated plants.

The minute galls produced by a partly attenuated culture of *Phytomonas tumefaciens* contained as high a concentration of thiamine per gram of dry weight as did the large galls produced by the virulent culture.

Stimulation to large gall production, where the attenuated culture was introduced into a stem below inoculations of the virulent culture, had slight if any effect on the thiamine concentration of the resulting galls.

Crystalline thiamine in lanolin failed to produce similar stimulation.

The bacterial cells of the partly attenuated culture, grown on a synthetic medium, contained more thiamine (22.1 gamma) per gram of dry weight than the cells of the virulent culture (17.5 gamma) grown on the same medium.

It appears, therefore, that thiamine alone does not have a causal role in crown gall initiation or development beyond that of any necessary food or growth factor transported to, or produced in, that area of meristematic activity.

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HARDWOOD INVASION IN PINE FORESTS OF THE PIEDMONT PLATEAU[1]

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INTRODUCTION

Forest stands of pure pine are important factors in the wood-using economy of the Carolina and Virginia Piedmont Plateau. Thousands of portable and semiportable mills representing everything from small, part-time, family-operated businesses to fairly large, full-time mills are, for the most part, dependent upon pine stumpage for their existence. Both the farm-woodland owner and the industrial landholder find that ready markets exist for a variety of pine forest products and that it takes hardwoods many more years to yield similar products. The demand not only for lumber but for pulpwood, poles, and a number of minor products has tended to call for a large proportion of pine in the total stumpage utilized locally. Although good grades of hardwood logs are usually accepted at the larger permanent mills, the market for these species, particularly oaks and hickories, has not been as steady as the market for pine. The large furniture industry of the piedmont region finds in nearby forests only a limited number of usable native hardwoods and is dependent on distant sources for sugar maple, birch, cherry, and other standard furniture woods.

Comparison of stumpage values and present growth rates of pines and hardwoods provides further evidence of the importance of pine species in piedmont forest enterprises. Records for 1940 show an average stumpage value of $5.83 per thousand board feet for 466 transactions involving a total of 204.2 million feet of yellow pine, principally shortleaf and loblolly, in the Carolinas and Virginia. From the upland hardwood type, dominated by oaks and hickories, a total of 4.2 million feet of oak stumpage was sold in 52 transactions at an average price of $5.73 per thousand board feet, while 258 thousand board feet of hickory in 9 transactions brought an average stumpage price of $4.16. Hardwood species that dominate the bottom-land hardwood type, on the other hand, have stumpage values equal to that of pine or even higher. Among these species are yellow poplar (*Liriodendron tulipifera*), sweetgum (*Liquidambar styraciflua*), red maple (*Acer rubrum*), black tupelo (*Nyssa sylvatica*), and sycamore (*Platanus occidentalis*), though markets for the last three are still quite limited.

Studies[2] of growth rates in existing stands indicate that pine types are much more productive than the upland hardwood type with which they are commonly intermingled. In the North Carolina piedmont, uncut second-growth sawlog-size stands occupy 25 percent

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[2] CRUIKSHANK, J. W. FOREST RESOURCES OF THE PIEDMONT REGION OF NORTH CAROLINA. U. S. Forest Serv. Appalachian Forest Expt. Sta. Forest Survey Release 6, 55 pp., illus. 1940. [Processed.]

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of the forest land. For the various forest types within this condition class, current annual growth per acre in board feet was found to be 300 for shortleaf pine, 331 for loblolly pine, 199 for bottom-land hardwoods, and 159 for upland hardwoods. Partly cut second-growth stands occupy about 18 percent of the forest lands; within this class, current growth was 189 board feet for shortleaf pine, 215 for loblolly pine, 202 for bottom-land hardwoods, and 124 for upland hardwoods.

Present evidence therefore indicates that the pine types not only grow at a much faster rate than the upland hardwood types, but also produce wood of greater value. Attainment of maximum returns from upland piedmont forests consequently appears to require maintenance of as high proportions of pine as are economically and silviculturally feasible. This is not necessarily true for the stream margins and other moist locations characteristic of the piedmont bottom-land hardwood type. Here growth is somewhat slower than in the pine types, but the lower production is offset by the greater values of some of the dominant hardwood species.

In the Carolinas and Virginia, the piedmont region, with a total land area of about 26 million acres, lies between the foothills of the Appalachian Mountains on the west and the level Coastal Plain to the east. A rolling, upland country, sloping gently eastward from elevations of 1,000 to 1,200 feet in the Appalachian foothills to 400 or 500 feet at its eastern margin, this region is favorable to agriculture because of its topography, a 6-months' growing season, and a 40- to 50-inch annual rainfall.

Long-continued and widespread farming has had a marked influence in modifying forest types and conditions. Slightly more than half of the land surface of this region is occupied by forest, of which more than 70 percent is classified as pine types. The cycle of land clearing, cultivation, soil erosion and impoverishment, and finally abandonment, has created conditions particularly favorable to the development of pure pine stands. Exposure of mineral soil, as in abandoned fields, is a requirement for the best germination and early survival of pine. The dispersal of the light, wind-borne seed from pines growing in adjacent forest completes the conditions under which the pines could gain ascendency over their common plant associates. Since three-fourths of the present forest area of the piedmont region was in cultivation at some time in the past, there is little reason to wonder at the high proportion of pine types now present.

During the past generation both land clearing and abandonment of land for farm-crop production have abated. Agriculture has become relatively stabilized on the better lands. Woods burning has been reduced by the direct activities of State and Federal forest-conservation agencies, and by the educational programs of these and other public and private organizations. Under such conditions natural trends in the development of forest associations might be expected again to become operative. For this reason the presence of numerous hardwoods in the understory of pure pine stands and their conspicuousness in the growth following logging have led many to suppose that nature tends toward stands of pure hardwoods or of mixed hardwoods and pine, rather than pure pine.

If quantitative evidence can establish that the understory hardwoods now present in pure pine types are thriving, then the assumed hardwood invasion can be more fully substantiated as an active trend already operative in the territory—a development which may seriously reduce the proportion of pine in future forests and have a marked effect upon silvicultural practices. Late in the summer of 1938 a study was made to collect quantitative data that would establish the widespread presence or absence of this trend and provide specific measurements of its character.

OBSERVATIONS BY OTHER WORKERS

Early literature dealing with the natural resources of the piedmont region provides strong evidence that the original forests were dominated by hardwood species. In 1858, Wilkes (*13*)[3] made especial reference to the culled-over oak forests of the piedmont and predicted the disappearance of hardwood timber there. Bruce (*3*) and Curtis (*4*) mentioned the predominance of oaks and hickories, the former stating that one-fourth of the original forests of Virginia were walnut. However, because hickory at that time was classed as a kind of walnut, it is assumed that a large proportion of this abundant "walnut" was *Carya* species. Hale (*5*) and Parkins (*8*) mentioned pine species as subordinate to hardwoods in old-growth stands. In 1897, Pinchot and Ashe (*9*) referred to the remaining original forest stands on piedmont uplands as being composed of oaks and hickories with an admixture of shortleaf pine in some places. Second-growth forests, however, had "pine for the forest body generally, and hardwoods as subordinate."

More recently, general observations by ecologists have led to the hypothesis that the extensive second-growth pine forests of the Piedmont are beginning to revert to hardwoods. Weaver and Clements (*10*), and Wells (*11*, *12*) have stated this point. Quantitative data substantiating the existence of such a trend have been published by Billings (*2*) who made detailed studies of seven old-field shortleaf pine stands occurring in Durham County. Even in the sandy soils of the longleaf pine type, Heyward (*6*) found strong evidence of replacement of pine by hardwoods where the forests had not been burned.

METHODS OF STUDY

The study was limited to stands of the two pines of the piedmont region most important commercially—shortleaf pine (*Pinus echinata*) and loblolly pine (*P. taeda*). A random selection of 40 piedmont counties in the Carolinas and Virginia was first made and sample stands within each of them were studied. It was necessary to exercise some choice of study areas in order to obtain samples of a wide range of stand ages, sites, and densities for both species. Sampling was further limited to areas where pine made up at least 90 percent of the overstory stem count and where neither grazing nor light cutting had taken place within 5 years, and no extensive cutting or thinning within 10 years. Bottom lands and swampy areas were excluded because they are not typical of the region and occupy a relatively small portion of the forest area. Within these limitations the selection of sample stands was objective, to prevent any partiality toward pine stands either with or without hardwood understories.

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[3] Italic numbers in parentheses refer to Literature Cited, p. 127.

In forest stands meeting the requirements named, detailed data were recorded on 117 plots (fig. 1). Of these, 65 were in the shortleaf type and 52 in the loblolly type. Most of the plots were one-fourth acre in size, but a few situated in dense, young stands were only one-tenth acre.

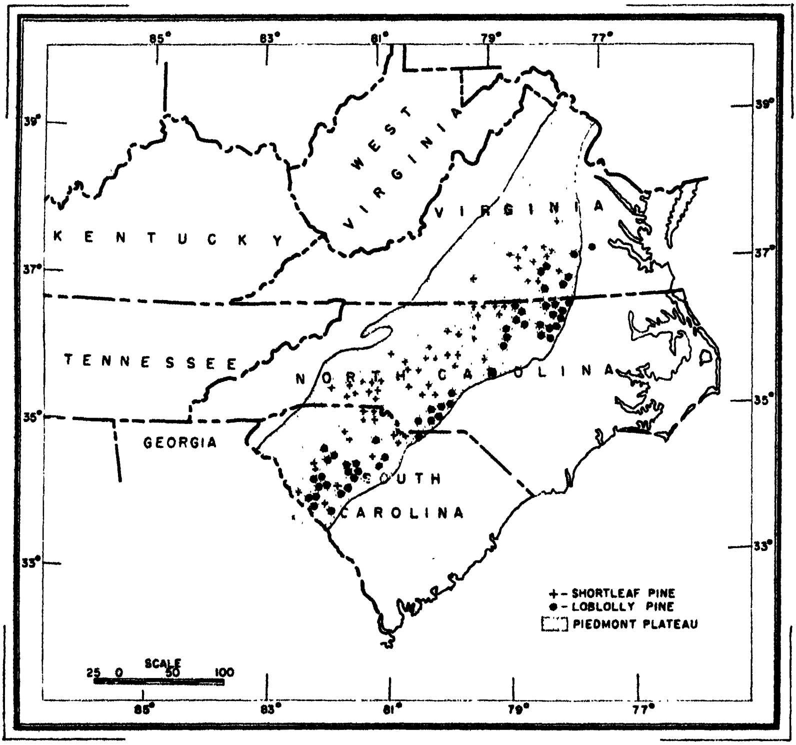

FIGURE 1.—Location of sample plots and extent of Piedmont Plateau in the Carolinas and Virginia.

On each sample plot a stem count of the woody vegetation, including both shrubby and arborescent forms, provided data for the three measures of the understory used, namely: (1) The percent of all hardwood stems represented by the climax species, oaks and hickories, (2) the number of oaks and hickories per acre, and (3) the number of stems of pine reproduction per acre. From measurements of sample trees, the pine overstory of each plot was described by age, site index, and density index. Methods of determining density of stocking in loblolly pine were those developed by MacKinney and Chaiken.[4]

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[4] MACKINNEY, A. L., and CHAIKEN, L. E. VOLUME, YIELD, AND GROWTH OF LOBLOLLY PINE IN THE MID-ATLANTIC COASTAL REGION. U. S. Forest Serv. Appalachian Forest Expt. Sta. Tech. Note 33, 30 pp., illus. 1939. [Processed.]

Density index is defined by these authors as the ratio (expressed as percent) of the observed number of trees of all species per acre to the number expected in fully stocked stands of loblolly pine. In the coastal region fully stocked loblolly stands of the following average diameters at breast height are assumed to contain trees per acre, respectively: 5.0 inches, 924; 10.0 inches, 283; and 15.0 inches, 142. Site index is defined as the average height in feet attained by dominant and codominant trees in such fully stocked stands at the age of 50 years.

For shortleaf pine, density of stocking was based on similar but as yet unpublished density criteria. The number of years having elapsed since the latest fire was carefully estimated from such evidence as the age of sprouts arising from fire-killed hardwood trees and the number of annual rings in the callus growth over fire scars found in cut stems.

The plots were then grouped into two categories: First (termed "unburned"), those which had not been burned over for 10 years or more; and second, those which had been burned at least once within 10 years. Data from each group were tested in a series of multiple regressions for relationships between measures describing the understory of each plot and measures describing the pine overstory. The multiple regression method was used because it provides a ready means for detecting the direction of trends and the testing of their significance. Graphic comparison of average regression lines with plotted residuals indicated that for most of the tests relationships were linear or nearly so. Because significant average trends rather than accurate predicting devices were sought, no attempts were made at refinements that would recognize the presence of curvilinear relationships in the few cases where this apparently occurred.

RESULTS FOR UNBURNED STANDS

The majority of the plots had not been burned within the preceding 10 years, and are referred to as unburned. It was from these unburned stands that the most significant and clear-cut results were obtained. Results from the burned stands were much less conclusive, probably because of the comparatively limited amount of data and the impossibility of reconstructing accurate fire histories.

Of the three criteria used to describe the pine stand—age, site index, and overstory density—the first is likely to be the most revealing in any study of long-time changes in the hardwood understory. This is clearly shown in table 1. The importance of the other two criteria lies in their conceivable effect on hardwood understories and hence on the correlation existing between age of the pine stand and the understory. Both density and site, however, as well as age, have significant effects upon understory pine reproduction. Such are the important generalities drawn from the study. They will be developed more fully in the following discussions.

Because oaks and hickories are commonly accepted as climax species of the region, their representation (percent of total stems) in the hardwood understories of pine stands is of particular significance. Furthermore, any consistent differences in their representation under pine overstories of varying age is strongly indicative of natural trends. Figure 2 shows that in unburned stands of both shortleaf and loblolly pine, oaks and hickories are abundant in hardwood understories, and also that their proportionate number increases with the age of the pine overstory. The climax species make up about 17 percent of the hardwood understory in 20-year-old shortleaf pine stands. This proportion rises steadily to approximately 42 percent in 90-year-old shortleaf. In loblolly pine stands the trend is the same but at a lower level. Strong evidence is thus provided that oaks and hickories are able to increase in competition with associated understory hardwoods.

TABLE 1.—*Summary: Correlation of dependent variables describing understory with independent variables describing pine overstory*

Dependent variable describing understory	Species and stand condition	Significant change with increase in overstory in respect to—		
		Age	Density index	Site index
Representation of oak and hickory stems in total hardwood understory.	Shortleaf:			
	Burned	None	None	None.
	Unburned	Significant increase.	do	Do.
	Loblolly:			
	Burned	do	do	Do.
	Unburned	Highly significant increase.	do	Do.
Number of understory oak and hickory stems per acre.	Shortleaf:			
	Burned	None	do	Do.
	Unburned	Highly significant increase.	do	Do.
	Loblolly:			
	Burned	do	do	Do.
	Unburned	do	do	Do.
Number of stems of pine reproduction per acre.	Shortleaf:			
	Burned	do	do	Highly significant increase.
	Unburned	Highly significant decrease.	Significant decrease.	Significant increase.
	Loblolly:			
	Burned	None	None	None.
	Unburned	do	Significant decrease.	Significant increase.

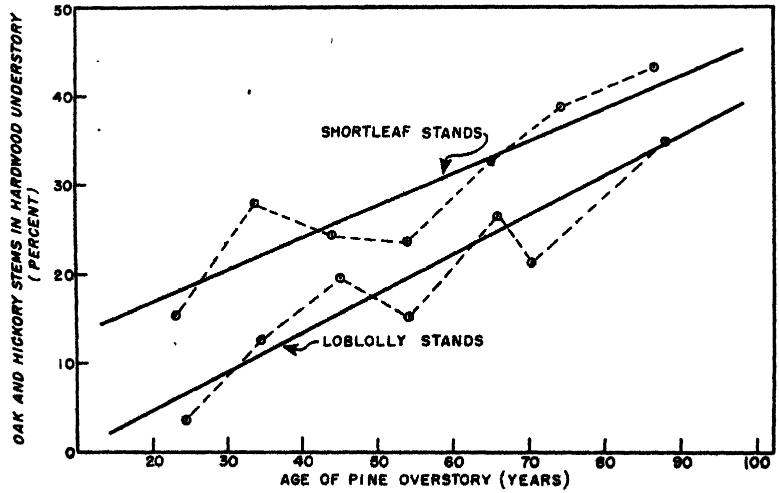

FIGURE 2.—Correlation between age of pine overstory and representation of oaks and hickories in the hardwood understory for stands unburned for at least 10 years. Plotted points are residuals.

Figure 3 shows that increasing representation of oaks and hickories is accompanied by an absolute increase in their numbers, in both shortleaf and loblolly pine stands. For the former the average number of understory oaks and hickories was about 200 per acre at 30 years of age, increasing to approximately 1,000 stems per acre at 90 years. For loblolly pine the average number of understory oaks and hickories was approximately 100 and 600 stems per acre at 30 and 90 years, respectively. These statistics showing absolute and relative increases in numbers of oaks and hickories substantiate the general observation that invasion by these climax hardwoods is now under way in the pine forests of the piedmont region.

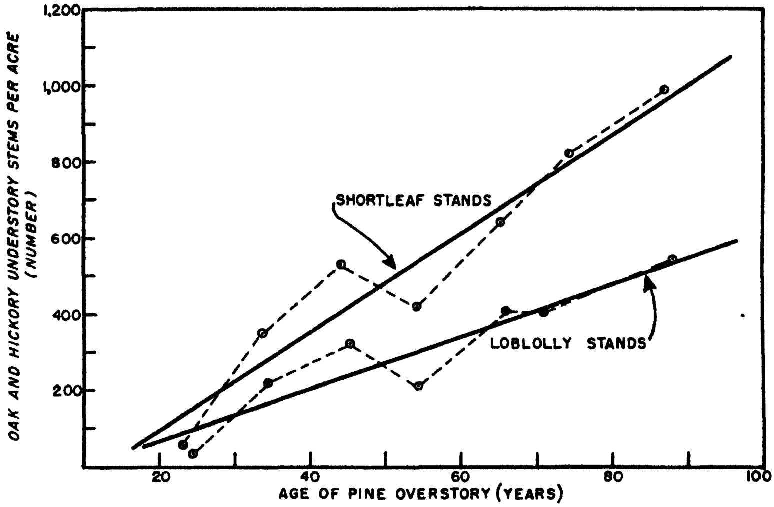

FIGURE 3.—Correlation between age of pine overstory and number of understory oak and hickory stems per acre for stands unburned for at least 10 years. Plotted points are residuals.

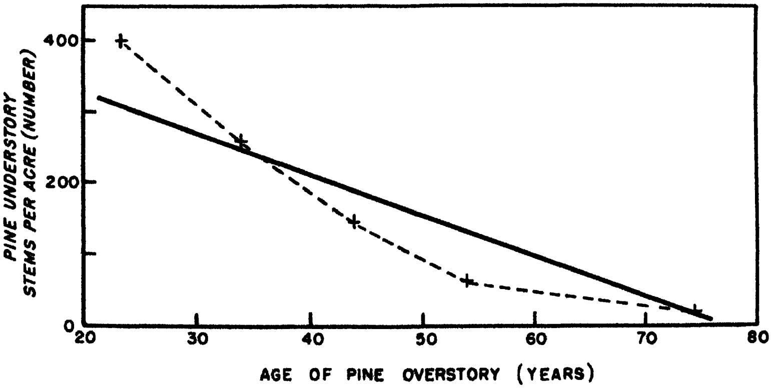

FIGURE 4.—Correlation between amount of understory pine reproduction per acre and age of pine overstory for shortleaf pine stands unburned for at least 10 years. Plotted points are residuals.

Relationships between pine reproduction and the pine-overstory characteristics already mentioned provide another measure of the importance of hardwood invasion. The pine reproduction showed a trend opposite to that of understory hardwoods. For shortleaf pine, figure 4 shows that the number of understory pine stems per acre decreases as the parent stand grows older and that average stands beyond 70 years of age contain very little pine reproduction. Figure 3 has shown that at this age 700 to 800 oaks and hickories per acre will be

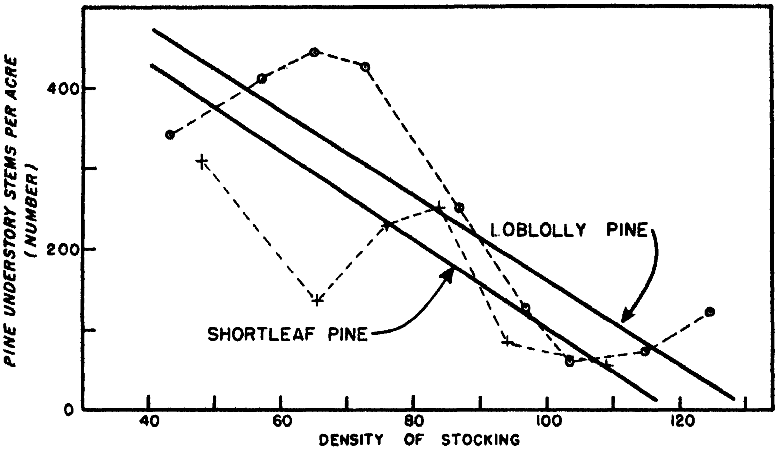

Figure 5.—Correlation between overstory density of pine stands and amount of understory pine reproduction per acre in stands unburned for at least 10 years. Plotted points are residuals.

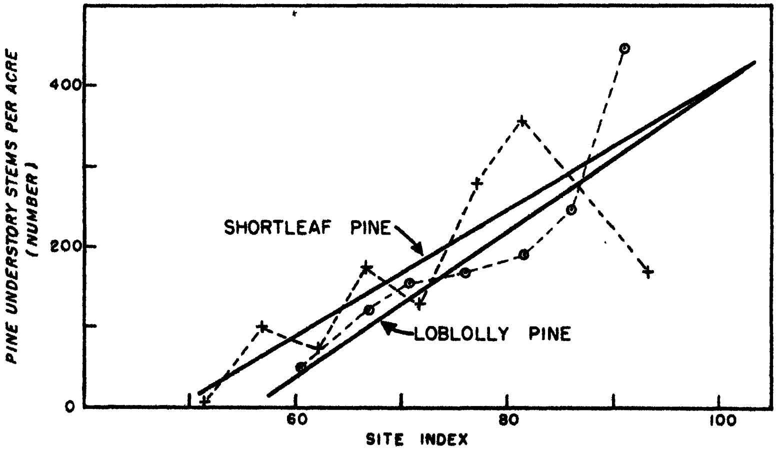

Figure 6.—Correlation between site index of pine overwood and amount of understory pine reproduction per acre for stands unburned for at least 10 years. Plotted points are residuals.

present. Understory reproduction of loblolly pine differed from that of shortleaf pine in its relation to age of the parent stand, since there was a slight increase in loblolly seedling count as the main-stand age advanced. From a statistical viewpoint, however, the amount of loblolly pine reproduction in unburned stands is not significantly correlated with overstory age (table 1). In other words, the average number of loblolly pine seedlings per acre does not differ greatly with respect to various ages of the overstory. For the stands studied, the average number per acre was approximately 200.

Aside from their differing relations with overstory age, the amounts of understory pine reproduction in both shortleaf and loblolly pine stands react similarly to variations in site index and overstory density. The reproduction of both species decreases sharply in numbers per acre as density of the overstory increases (fig. 5). This gives further evidence of the intolerance to shade of these species and their general disinclination to grow in uneven-aged stands except those which are very open. Figure 6 shows that this trend may, however, be modified by site, the amount of reproduction of each species increasing with the site index.

Discussion of the development of understories in relatively unburned pine stands has thus far been confined to average trends. More specific comparisons of understory conditions for various combinations of overwood age, site, and density are presented in figure 7.

For shortleaf pine, figure 7 indicates that in stands 40 to 50 years of age and older, the climax hardwoods will outnumber pine reproduction except under the lower overwood densities. It is also apparent that the excess of climax hardwoods over pine reproduction is likely to be greater on the poorer shortleaf sites than on the better ones. The Forest Survey has shown that for shortleaf pine stands in North and South Carolina, site index 60 is the one of most frequent occurrence. It follows that the advance reproduction in stands of sawlog size, say those older than 45 years, is quite likely to be dominated by oaks and hickories rather than by the reproduction of the parent stand.

For loblolly pine the trends are somewhat different. The number of understory stems of this species is more nearly equal to the number of oaks and hickories. Nevertheless, on sites 60 and 70 oaks and hickories outnumber pine reproduction in the better-stocked older age classes. According to Forest Survey records, site index 70 is of most frequent occurrence in loblolly pine stands of the Carolinas. Consequently, advance reproduction dominated by climax hardwoods may be commonly expected in many of the better-stocked loblolly stands, although figure 7 indicates that the preponderance of oaks and hickories over pine in the understory will not be so great in loblolly as in shortleaf pine stands. On loblolly pine sites 80 and 90, particularly the latter, the amount of pine reproduction exceeds that of climax hardwoods except in the oldest and densest stands.

In the foregoing discussion comparisons have been made between the amounts of pine reproduction and climax hardwoods present in the understories or advance reproduction of pine stands. The climax hardwoods, oaks and hickories, were used in these comparisons because it seems reasonable to expect that pine reproduction will suffer more severe and persistant competition from them than from secondary hardwoods.

Although for certain combinations of overwood conditions, as already shown, oaks and hickories do not exceed pine stems in the understory, when the number of secondary hardwoods is added (fig. 2) the total is much greater than that for both pine species for practically

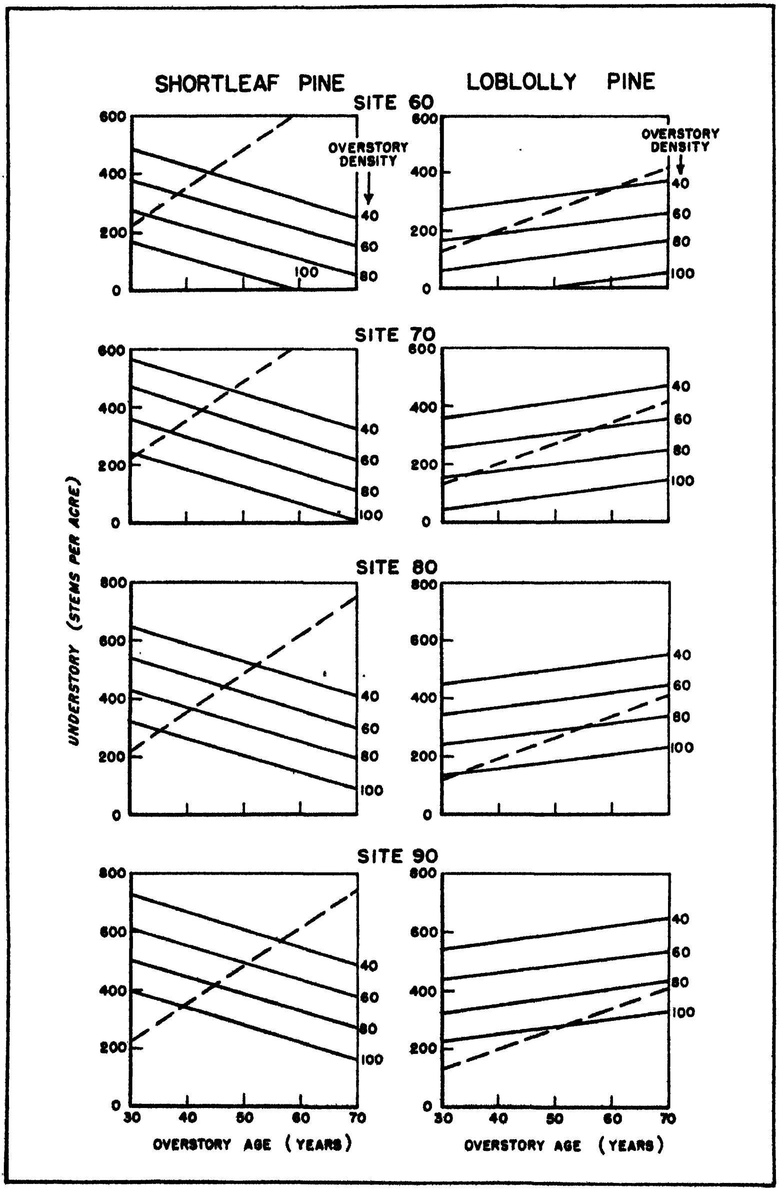

FIGURE 7.—Comparison of understory pine with understory oak and hickory stems in pine stands for various combinations of overwood age, density, and site. Broken lines show average numbers of oak and hickory stems, solid lines average numbers of pine understory stems. Stands unburned for at least 10 years.

all overstory combinations. Since the competition of secondary species may fully equal that of the oaks and hickories in limiting pine establishment and growth during the first few years after cutting, the combined effect of primary and secondary hardwoods is of first importance.

RESULTS FOR BURNED STANDS

It is interesting to compare the foregoing evidence of successional trends in the relative absence of fire with findings in stands burned at least once during the past 10 years. Because sample-plot records from such burned stands were available only in limited quantity, however, and because both fire histories and intensity of individual fires probably varied greatly, few significant relationships were discovered.

As shown in table 1, no evidence of hardwood succession in burned stands of shortleaf pine was found. Neither the number of stems nor the percent of climax species in the understory was correlated with overstory age, density, or site. Apparently the fires in these shortleaf stands had the effect of halting the trend toward hardwoods. Climax species in the understory were not eliminated, however, but were present on an average in about half the numbers found in unburned stands. Pine reproduction in the burned stands, it may be added, was more abundant on the better sites, as it was also in the unburned stands.

The burned-over loblolly pine stands studied gave evidence of successional trends similar to those in unburned stands. Both the proportion of climax species in the understory and their absolute numbers were found to increase significantly as the pine overwood grew older. Moreover, in these respects there was almost no difference between burned and unburned stands. Figures 8 and 9 when compared with figures 2 and 3 illustrate this result. Evidently the fires that occurred in the loblolly stands had very little effect on the understory hardwoods.

Effects of fire on understory pine reproduction also differed between stands of the two pine species. In shortleaf pine stands, the number of understory pine stems was found to increase as the overwood grew older. This relationship, shown in figure 10, is in direct contrast to the trends of understory pine reproduction in unburned stands, as may be seen by referring to figure 4.

In burned loblolly pine stands all normal relationships between amount of pine reproduction and overstory characteristics appeared to be upset by the fires. Pine reproduction had not been eliminated in these burned stands, but was present in from one-half to two-thirds the amounts found in similar unburned stands.

To summarize the results from the burned stands, it can merely be stated that fire was apparently favorable to maintenance of shortleaf pine, but showed opposite effects in loblolly stands. No evidence gathered in the study was helpful in explaining this inconsistency. It is believed that the shortleaf and loblolly pine stands examined had generally dissimilar fire histories, and that the differences in fire effects are due primarily to such variations. It did not appear possible to reconstruct the fire histories accurately by the ex post facto procedures necessarily used.

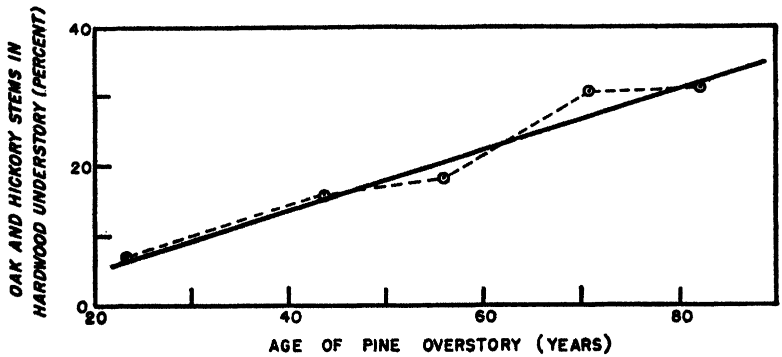

FIGURE 8.—Correlation between age of pine overwood and representation of oaks and hickories in the understory for loblolly pine stands burned at least once during the past decade. Plotted points are residuals.

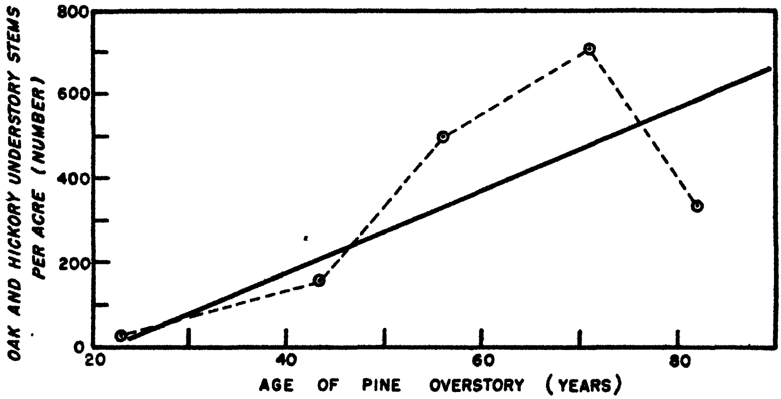

FIGURE 9.—Correlation between age of pine overstory and number of understory oak and hickory stems per acre for loblolly pine stands burned at least once during the past decade. Plotted points are residuals.

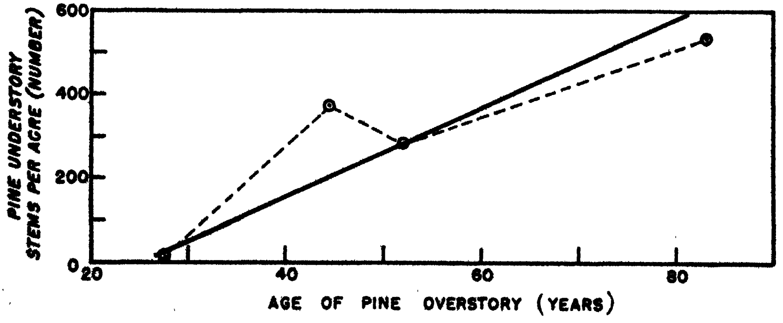

FIGURE 10.—Correlation between age of pine overstory and amount of understory pine reproduction per acre for shortleaf pine stands burned at least once during the past decade. Plotted points are residuals.

IMPLICATIONS OF RESULTS IN FOREST MANAGEMENT

The establishment of hardwood invasion as an aggressive successional trend in shortleaf pine stands and a less aggressive but nevertheless distinct trend in loblolly pine carries far-reaching implications with respect to the management of these stands. Not only do the oaks and hickories in the understory outnumber the pines on many unburned areas, but these climax species are only a part of the total number of understory hardwoods. Many of the associated hardwoods play a subordinate role in mature hardwood stands, but they may be equally effective competitors of pine in juvenile stages. Not only do these hardwoods compete with smaller numbers of growing pine seedlings for light, moisture, and nutrients, but their presence creates a condition unfavorable to the ready establishment of pine reproduction. The mantle of hardwood leaf litter tends to prevent contact of the pine seed with the mineral soil, which is essential for good germination and early survival of the pines. On the other hand, a litter of hardwood leaves forms the best natural seedbed for good germination and early survival of heavy-seeded species such as the oaks (*1*, *7*).

Although it is entirely probable that it will be advantageous to allow replacement of pine to take place on some areas, it is at present impossible to determine which these are, except for the more obvious locations in well-drained stream bottoms and other moist sites where the more valuable hardwoods are usually present in some degree. Investigation of soil characteristics, which have been markedly changed by several generations of cultivation and subsequent depletion, probably offers the best avenue for solution of this problem.

It is also probable that no practicable forest-management measures can entirely control a natural trend so pronounced and persistent as the one described. The drastic measures that originally accomplished control of hardwoods and creation of pure pine stands, namely, extensive land clearing and cultivation, cannot be considered practical in timber production. It is possible, however, that workable methods for partial control of hardwoods and maintenance of high proportions of pine can be developed.

One possibility that suggests itself is the use of fire. Controlled burning, for a variety of purposes, has long been a controversial subject in the South. Although this study provides some indication that fire may favor establishment of pine, the evidence is not at present sufficiently clear-cut and consistent to justify recommendations of burning as a general practice.

For the immediate future, the most promising measures for maintenance of a high proportion of pine in mixed stands are found in the methods of taking the final harvest and in the silviculture applied to subsequent new growth.

Where forestry is practiced, two general methods are customarily considered for harvesting mature pine saw timber, i. e., extremely heavy or clear cuttings by the strip, spot, or seed-tree method, and lighter or selective cuttings. Following seed-tree cuttings, no trees remain except the few chosen, and all or part of these may be removed after reproduction is established. In clear cutting, it is expected that renewal of the stand will be accomplished by seed already on the ground or dispersed from uncut bodies of timber purposely left adjacent to the cutting area. The objective of the clear-cutting method is to produce even-aged stands so distributed that annual or periodic harvests of financially mature portions of the forest represent a sustained yield from a given property. The method envisions thinnings, improvement cuttings, and salvage cuttings prior to the final cut, for the dual purpose of obtaining intermediate harvests and maintaining the most desirable growth rate of those stand components chosen for the final crop. The basic concept of this method, therefore, is the management of a forest property by even-aged groups and it is usually thought to apply best to species that do not reproduce well in the shade of older trees. The term "clear cutting" is understood to connote the orderly harvest of mature stands with area regulation to keep the rate of cutting within the limits of sustained yield, and carefully planned provision for reproduction.

In the lighter or selective cuttings, individual mature or poor-risk trees are removed throughout an entire cutting area and a comparatively heavy stand remains. The method usually combines essential features of both harvest and improvement cuttings in a single operation, which is repeated at short intervals. Here, renewal of the stand is accomplished by seedling establishment in the small openings created by the selective cutting. The usual concept among foresters is that the use of this method implies the maintenance of an all-aged or many-aged forest, wherein several or many age classes may be present on an area as small as a quarter or even a tenth of an acre. The method is commonly considered as being the best for optimum long-time production of shade-tolerant species. As used here, therefore, the term "selective cutting" applies only to those operations by which mature saw timber is harvested and provision is made for regeneration under a silvicultural policy aimed at development and maintenance of all-aged stands. It does not include thinnings and improvement cuttings made in young stands for intermediate harvests of pulpwood, poles, or piling.

Will these two methods provide equally well for a high proportion of pine in future forests, where aggressive hardwood invasion exists? In the all-aged stands produced by selective cutting it is probable that the loblolly and shortleaf pine overstory would average about 40 in density index and about 70 years in age. The most common site index in shortleaf pine stands is 60. For site index 60, density index 40, and age 70, figure 7 shows that the average understory will be composed of about 250 pine seedlings and 740 oak and hickory stems per acre. Figure 2 shows that oak and hickory stems will make up about 35 percent of all understory hardwoods; accordingly the total number of hardwood stems will be more than 2,100. For loblolly pine of the most common site, which is 70, there will be about 460 pine seedlings and 410 oaks and hickories per acre in the average understory. Figure 2 shows that these climax species will be about 27 percent of all understory hardwoods, totaling 1,500 stems per acre. With hardwoods so far outnumbering pine in the understory, the chances that pine seedlings will occupy each opening made by selective cutting appear poor indeed.

If overstories are maintained at densities higher than index 40, the average amount of understory pine reproduction will be reduced, with the possibility of higher odds against the filling of the openings by the growth of pine seedlings. If pine overstories are maintained at densities below index 40, the amount of understory pine reproduction increases with probable consequent improvement in the chance that some pine seedlings will grow through the hardwoods into dominant positions. But as overstory densities are reduced the cuttings depart further from the usual concept of selective cutting and approach the more drastic treatments that result in production of even-aged stands.

For the common pine sites of the piedmont region, therefore, this study indicates that selective cutting in pine saw-timber stands may promote the gradual replacement of pine with hardwoods. Because pine reproduction is better represented in understories of stands with low overwood densities and because it is generally conceded that both shortleaf and loblolly pine seedlings develop more rapidly in full light than in shade, it becomes necessary to consider the possibility that some form of drastic treatment, such as clear cutting by the strip, group, or seed-tree system, is more likely to maintain high proportions of pine in future forests than is selective cutting. Clear cutting brings about certain physical conditions that may reasonably be expected to favor maintenance of pine. The complete felling of the heavy pine overstory breaks down a portion of the hardwood understory present. For the removal of the comparatively high volume of logs, more swamping, skidding, and hauling are necessary, still further reducing the hardwoods. These operations also break up the mantle of forest litter and expose mineral soil, creating conditions favoring the germination of pine seed. Complete removal of overhead shade and the reduction of root competition will accelerate the growth of pine after establishment. Under the conditions created by such operations, the new growth of pine starts off on a more equal basis with the hardwoods than when selective cutting is practiced.

When a seed-crop failure occurs in the autumns preceding or immediately following the harvest cut, so that there is insufficient pine reproduction, the hardwoods may get a start of 2 or 3 years. After the establishment of seedlings from a subsequent cone crop, a cleaning several years later to release pine crop trees from hardwood competition may be essential.

Two kinds of stands encountered in shortleaf and loblolly pine types are possibly not in danger of hardwood invasion. One of these is loblolly pine on an unusually high-quality site, and the other occurs in areas where apparently an unusual series of repeated light fires over a long period of years has so reduced hardwoods that their invasion will be very slow. It has already been shown that on loblolly pine sites of 90 or more, the understory will contain a fair percentage of pine seedlings, which will outnumber climax hardwoods except in fully stocked stands of the older age classes. General observation indicates that here pine is frequently able to penetrate through and keep above the hardwood understory. Under these conditions it is possible that either even-aged or all-aged management may maintain the pine in high proportions.

In the second type of stand, where hardwoods have been largely eliminated by light fires, it is again probable that either type of management will maintain high proportions of pine for some time, particularly on the better sites. Under such conditions there will be few, if any, hardwoods to compete with pine seedlings.

Except for the two conditions discussed above, the evidence presented here points to a likelihood that clear-cutting measures will prove superior to selective cutting, where maintenance of high proportions of pine is an objective of management.

SUMMARY AND CONCLUSIONS

Conclusions based on results in stands unburned for at least 10 years may be summarized as follows:

(1) In both loblolly and shortleaf pine stands, the proportion of climax species, oaks and hickories, in hardwood understories increases with age of the pine overwood.

(2) The number of understory climax hardwoods per acre also increases with age of the main pine stand.

(3) Neither the density nor the site index of the pine stand has a significant influence upon the two trends mentioned above.

(4) In shortleaf pine stands, the amount of pine reproduction per acre decreases significantly as the overstory grows older, but under loblolly pine it tends to remain approximately constant for all ages of the overstory.

(5) In both loblolly and shortleaf pine stands the amount of understory pine reproduction per acre decreases with increasing density of overwood, but increases with improvement in the site index.

(6) Differences in age, density, and site of both loblolly and shortleaf pine overstories are associated in several different ways with the amounts of pine reproduction present in the understories. Under certain overstory stand conditions the oaks and hickories will outnumber pine reproduction, and under other conditions the reverse will be true, as shown in figure 7.

(7) The number of combinations of overstory age and density under which climax hardwoods will exceed the amount of pine reproduction is greater on poor than on good sites for both species of pine. In shortleaf the preponderance of climax hardwoods over pine reproduction is greater than in loblolly stands. Hence the trend toward replacement of pine by oaks and hickories appears to be the more aggressive in shortleaf pine stands.

(8) For site index 60 (fig. 7), which most frequently characterizes shortleaf pine stands of the Carolinas, oaks and hickories will outnumber pine reproduction with respect to the majority of possible combinations of overwood age and density. For site index 70 (fig. 7), of most frequent occurrence in loblolly pine stands, oaks and hickories will outnumber pine reproduction only in stands of higher densities.

(9) Climax oaks and hickories represent only a part of the entire hardwood understory of both shortleaf and loblolly pine stands. Figures 2 and 7 indicate that in most of these stands pine stems in the understory will be greatly outnumbered by hardwoods, counting not only the climax species but also the secondary hardwoods, which may be effective competitors of pine during the reproducing years following cutting.

In stands burned at least once during the past 10 years the findings were as follows:

(1) In the burned shortleaf pine stands studied, understory climax hardwoods were present in about half the amounts found in unburned stands. No tendency toward an increase in the representation or numbers of oaks and hickories was found.

(2) Understory pine reproduction in burned shortleaf pine stands showed a marked increase with advancing age of the overstory, a reversal of the trend found in unburned stands.

(3) Climax hardwoods in the understories of loblolly pine stands showed the same trends as those in unburned stands. Fires in loblolly stands apparently had little effect upon the numbers and representation of understory oaks and hickories.

(4) Pine reproduction, although not eliminated, was reduced in burned loblolly stands, and the pine understory had no consistent relationships with overstory density and site.

(5) The study failed to disclose any reasons for the inconsistencies in results for burned stands. Lack of consistency is attributed primarily to wide but undetermined variations in fire histories of the stands studied.

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LIFE HISTORY AND DISTRIBUTION OF PYTHIUM AND RHIZOCTONIA IN RELATION TO DAMPING-OFF OF RED PINE SEEDLINGS[1]

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INTRODUCTION

Damping-off continues to influence production in Wisconsin forest nurseries. Serious outbreaks are sporadic but small losses are experienced each year. The unpredictable occurrence of epidemic attacks in the various nurseries is a handicap to the sustained production demanded by the extensive planting program. Losses are less severe on newly cleared land, but economy is requiring the continued use of land already improved. Results with available control measures on infected land are highly variable, both between different localities in the same year and in the same localities during different years. Various modifications of cultural practices fail consistently to control the disease. The common soil treatment with sulphuric acid, while often successful in controlling damping-off, may both cause toxic injury to the seedlings and produce undesirable disturbances (*20*)[3] in the nutrition and biology of the soil.

The extensive literature of well-known damping-off diseases of conifer seedlings has been reviewed elsewhere, e. g., by Hartley (*7*), C. Roth (*15*), Ten Houten (*18*), and L. F. Roth.[4]

The causal agents described by the various workers have commonly been *Pythium* spp. and *Rhizoctonia solani* Kühn, with species of *Fusarium* and *Phytophthora* active in certain localities. With exceptions noted later, most earlier work has been on etiology and control measures with relatively little attention devoted to epidemiology.

In the present series of studies the basic information essential to intelligent development of control measures for Wisconsin has been sought. This paper deals with the identification of the causal fungi,

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[3] Italic numbers in parentheses refer to Literature Cited, p. 147.

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the symptoms they induce, their life histories in relation to pathogenesis, and their distribution. In subsequent papers are considered the influence on damping-off of temperature, moisture, and soil reaction (*16*) and the seasonal development of the disease in the nursery (*17*).

CAUSAL ORGANISMS

ISOLATION

The causal fungi were readily isolated from damped-off seedlings. During the fall of 1936, in the greenhouse, red pine (*Pinus resinosa* Ait.) seed was planted in infected Plainfield sand from the nursery at Wisconsin Rapids. Typical damping-off appeared 3 days after emergence of the first seedlings. A suitable section was removed from the diseased hypocotyl of each damped-off seedling, sterilized for 3 minutes in 1-to-1,000 mercuric chloride, washed twice in sterile water, and plated on potato agar. *Pythium* cultures were purified by subsequent transfers of hyphal tips through water agar. The fungi most commonly obtained were: (1) Phycomycetes, resembling *Pythium*, (2) several species of *Fusarium*, and (3) a few cultures resembling *Rhizoctonia*. The studies on pathogenicity, cultural characters, and morphology were originally made on the 2 dozen cultures thus secured and checked by numerous subsequent isolations.

PATHOGENICITY

The pathogenicity of certain cultures upon red pine was tested in the greenhouse. Four-inch pots of Plainfield sand from the nursery were steamed for 20 minutes at 15 pounds pressure. While still hot, each pot was placed on a clean bench in a section of a sterile Petri dish and was fitted with a cover consisting of a truncated celluloid cone plugged at the top with nonabsorbent cotton. Twenty-four cultures were tested. Four pots were allowed for each culture and 8 pots for controls. For soil inoculation, cultures on potato agar in Petri dishes were cut into 4-mm. squares. Six squares were spaced uniformly on the soil surface of each pot. Control pots received squares of sterile agar. Each pot was seeded with 25 washed red pine seeds placed at the same level as the inoculum. Seed and inoculum were covered three-sixteenths of an inch deep with steamed nursery soil. When necessary, distilled water was added from below. At 3-day intervals after emergence all damped-off seedlings were removed with sterilized forceps, and the number from each set of 4 pots was recorded. The counts were continued for a month, when the seedlings had reached an age of practical immunity. The results are given in table 1.

All cultures of *Pythium* and *Rhizoctonia* were pathogenic on red pine. The *Rhizoctonia* cultures damped-off an average of about 60 percent of the seedlings and ranged from 23 percent (F–3) to 95 percent (F–118). *Pythium* cultures also averaged 60 percent but ranged from 36 (F–10) to 87 (F–111–A) percent. These results correspond with Hartley's (*7*) report that ". . . the variation in virulence between the different strains of *P. debaryanum* on pine seedlings is less than the variation in strains of *Corticium vagum*." Hartley[6] now considers that the fungus on which he reported was not *P. debaryanum* and probably was the same as that used in the present studies. The *Fusarium* strains here isolated were apparently of little importance in causing damping-off of red pine. Isolations in every case yielded the fungus used for inoculation.

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[6] Correspondence.

TABLE 1.—*Pathogenicity of single hyphal tip cultures of damping-off fungi upon red pine in steamed soil*

Fungus	Culture No.	Seedlings emerged from 100 seeds	Damping-off			
			Post-emergence	Pre-emergence [1]	Total	
		Number	*Number*	*Number*	*Number*	*Percent* [2]
Pythium	F-1	52	28	35	63	72
	F-7	63	9	24	33	38
	F-8	46	19	41	60	69
	F-9	70	16	17	33	38
	F-10	78	22	9	31	36
	F-11	66	18	21	39	45
	F-13	48	20	39	59	68
	F-20	55	28	37	65	75
	F-22	73	35	14	49	56
	F-111	60	26	27	53	61
	F-111-A	42	31	45	76	87
	F-117	49	26	38	64	74
Fusarium	1	86	0	1	1	1
	2	[3] 88	0	0	0	0
	3	86	1	1	2	2
	4	[3] 90	2	0	2	2
	5	85	0	1	1	1
	6	87	1	0	1	1
	7	86	0	1	1	1
Rhizoctonia	F-2	51	18	36	54	62
	F-3	78	3	17	20	23
	F-5	57	21	30	51	59
	F-8	62	26	25	51	59
	F-118	23	19	64	83	95
Control	(4)	86	0	......	0	0
	(4)	88	1	......	1	1

[1] Numbers represent germination in controls less germination in inoculated pots.
[2] Percentages represent total damping-off in inoculated pots expressed as percentage of seedlings emerging in controls.
[3] Emergence slightly greater than average for the controls may be attributed to variability in germinating capacity of seed.
[4] No inoculation.

IDENTIFICATION

The characteristic vegetative growth and reproductive structures formed on plant tissue in clear agar readily placed the phycomycetous isolates in the genus *Pythium*. Five representative cultures were identified by Charles Drechsler, of the United States Department of Agriculture, as *Pythium irregulare* Buisman. This fungus produces many spherical to pyriform sporangia and later numerous spherical oögonia that in many cases bear several projections of various sizes. Both smooth and irregular oögonia are found on the same thallus. This fungus is obviously closely related to *Pythium debaryanum* Hesse but differs in possessing the occasional oögonial projections. The writers have found no report of *P. irregulare* causing damping-off of conifer seedlings. Buisman (*2*) originally isolated *P. irregulare* from decaying roots of peas and lupines and from cucumber seedlings which failed to emerge. Matthews (*10*) isolated it from a decaying gametophyte of *Anthoceros* and from damped-off tomato plants.

The strains of *Rhizoctonia* represented by cultures F-5 and F-118, and a number of isolates with the same cultural characteristics as F-119 were all isolated from damped-off conifer seedlings grown in Plainfield sand. Culture F-119, however, was obtained from a sclerotium on a potato tuber grown in Plainfield sand. To corroborate the morphological evidence regarding specific rank of the *Rhizoctonia* cultures, in a single test, the three strains were inoculated into incisions in young potato sprouts and into sterilized soil seeded with cabbage and with red pine. All of the three cultures produced lesions on potato shoots, but only F–118 and F–119 attacked the cabbage seedlings. Cultures F–118 and F–5 caused severe damping-off of pine seedlings while F–119 was not pathogenic. It appeared that the *Rhizoctonia* cultures were strains of *Rhizoctonia solani.*

The morphology of the several isolates of *Rhizoctonia* studied conformed closely to that given by various workers (*14*, *3*, *12*) for strains of *R. solani.* When grown on potato-dextrose agar they fell into three distinct cultural groups: (1) Cultures typified by F–118 appeared distinctly mealy with abundant, short, minutely tufted hyphae. Growth was close to the substrate, and there was a tendency toward zonation. Aerial mycelium was light-colored, the colony appearing drab (*13*). No pigment was formed in the substrate, and no sclerotia were produced. (2) Cultures resembling F–119 appeared silky with long, straight hyphae radiating from the point of inoculation. Tufted mycelium was sparse, zonation lacking, and growth was close to the substrate. The cultures were drab but not so light as F–118. No pigment was formed in the substrate. A true sclerotium 1 to 3 cm. in diameter invariably formed at the point of inoculation, and small sclerotia appeared in contact with the glass at the edge of the Petri dish. All sclerotia were closely appressed to the substrate. (3) Cultures typified by F–5 had, in addition to the radiating surface mycelium, a dense interlacing web of aerial hyphae over the colony. Many dark brown mycelial tufts 0.5 to 2.0 mm. in diameter, made up of short, barrel-shaped cells, were enmeshed in the aerial mycelium and at the surface of the substrate. The culture was distinctly zonate. The mycelium was dark brown, the colony appearing bister (*13*). Some brown pigment was formed in the substrate. Though the above mycelial tufts appeared like sclerotia, they did not become hard or homogeneous like the sclerotia of F–119.

SYMPTOMS CAUSED BY PYTHIUM AND BY RHIZOCTONIA

Characters of moderate diagnostic value are known in relation to the distribution of *Pythium* and *Rhizoctonia* damping-off in the conifer seedbeds, *Pythium* commonly occurring in a roughly random manner, but *Rhizoctonia* appearing in definite fair-sized patches within which most or all seedlings are killed. However, differential symptoms on individual seedlings have not been found by the writers in the several comprehensive descriptions (*9*, *7*, *15*) of conifer damping-off. On Plainfield sand, where *Pythium* and *Rhizoctonia*, respectively, were the fungi concerned, and where the seedlings were examined at suitable stages of development, the writers found that the two fungi produced distinctive symptoms in the same host. Though most observations were on red pine, this condition appeared also for jack (*Pinus banksiana* Lam.), eastern white (*P. strobus* L.), and Austrian (*P. nigra* Arn.) pines.

The following descriptions are based on an examination of seedlings of all ages damped-off (1) in the greenhouse in disease-free soil inoculated with identified pure cultures and in naturally infected soil and

(2) in the nursery in inoculated soil and in naturally infected soil. The seedlings were grown over wide ranges of environmental conditions. The symptoms were checked by isolations of the causal fungi in several hundred cases. The details of these series of studies are omitted because of their volume.

Roots of seedlings less than 2 weeks old were attacked by *Pythium* at any point throughout their length. Older roots became infected in their younger parts. Seedlings from soil containing *Pythium* showed all degrees of infection from lesions bordering on microscopic visibility to a buckthorn-brown (*13*) discoloration of the entire root except the remote tip involving the growing point. This discoloration of the root was the first symptom of *Pythium* damping-off and became more or less complete before the disease appeared above the soil surface (fig. 1, *A*). In contrast to the hypocotyl, which later became infected, roots in the early stages of destruction might be entirely discolored and water-soaked and yet appear firm and distended. They showed no tendency to shrivel, and since the above-ground parts appeared normal, they evidently continued to absorb water.

Infection of the hypocotyl was by growth of the fungus upward through the primary root and therefore was not apparent until relatively late in the course of seedling destruction. The base of the hypocotyl of seedlings attacked by *Pythium* was commonly a shade of green either lighter or darker than the normal plant green. The intensity of this color appeared to be influenced by seedling age and environment. The red pigments completely disappeared from the infected areas but were unchanged in the upper, uninfected parts. During the early stages infected tissues were water-soaked, translucent, and not greatly shrunken. However, even with abundant moisture the advanced lesions became shriveled.

Initial attack by *Rhizoctonia* was most frequent at the base of the hypocotyl or root crown. Within the seedling the fungus advanced more slowly than did *Pythium* and appeared to grow either up or down the main axis with equal facility. Attacked seedlings, therefore, showed typical above-ground damping-off symptoms relatively early in the course of the disease. The lower parts of roots on such seedlings were commonly found to be white and healthy (fig. 1, *C*, *D*).

Chlorophyll, as well as the red pigment, was destroyed in tissues attacked by *Rhizoctonia*. The red pigments were either formed anew or translocated from their original position in the base of the hypocotyl up to the originally green top. The predominant color of fresh lesions was a dark, bluish gray-green. Lesions on the hypocotyls of seedlings near the age of seed-coat shedding became bleached almost to a straw color. These infected parts then appeared dry and fluted and later became markedly shriveled. Seedlings attacked by *Rhizoctonia* when only the hypocotyl curve extended above the ground were usually olive-green in color and often appeared even a little inflated. Examination of seedlings of all ages attacked by *Rhizoctonia* showed numerous mycelial strands extending from the soil to the seedling. When such seedlings were pulled from the ground, a small quantity of soil was commonly found laced to the stem by the superficial mycelial web.

While symptoms caused by *Pythium* and *Rhizoctonia*, respectively, were distinct, the presence of other damping-off fungi, invasion by secondary organisms, or the lapse of too long a time after infection

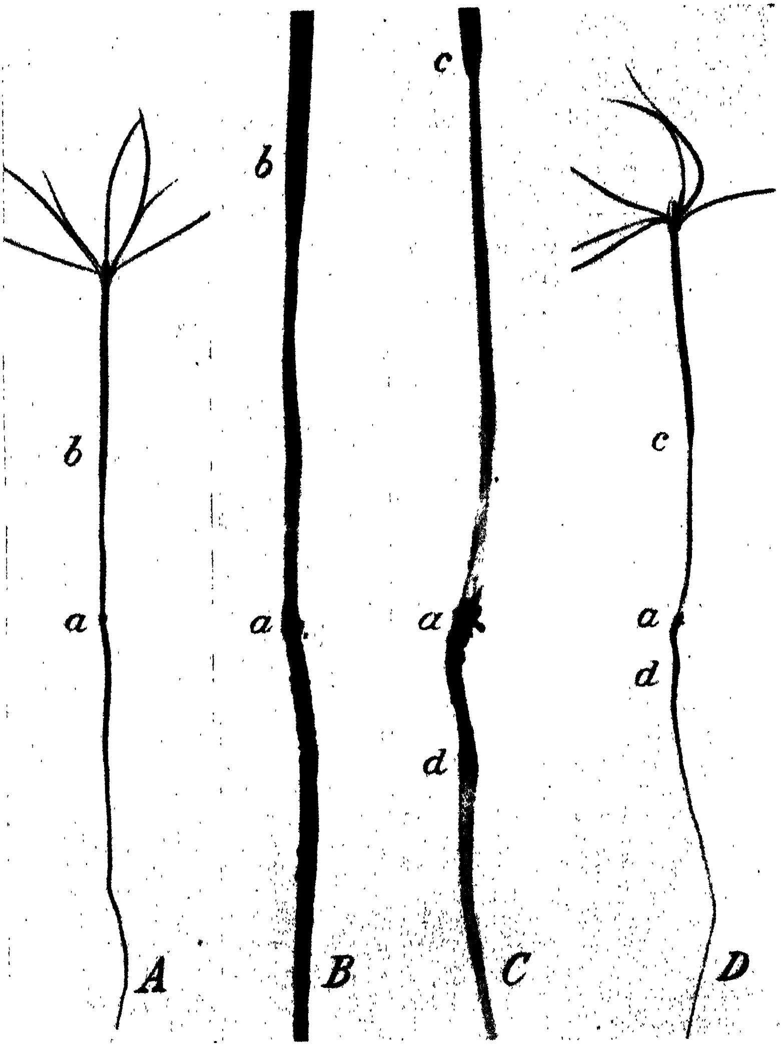

FIGURE 1.—Symptoms caused by *Pythium* (*A* and *B*) and *Rhizoctonia* (*C* and *D*) on 2-week-old red pine seedlings. *B* and *C* are, respectively, 3× enlargements of *A* and *D*. In every case the soil line is at *a*. In the case of *Pythium* (*A* and *B*) all the tissue below *b* is diseased, that from *b* to *a* is water-soaked and dark green, and that below *a* is water-soaked and brown. In the case of *Rhizoctonia* (*C* and *D*) the tissue from *c* to *d* is diseased; that from *a* to *c* is straw-colored, dry, and somewhat shriveled; that from *a* to *d* is brown and has probably been invaded by secondary fungi; that below *d* appears healthy.

could obscure the typical *Pythium* and *Rhizoctonia* symptoms. Therefore, field identification had to be approached with caution.

LIFE HISTORIES IN RELATION TO PATHOGENESIS

Certain phases of the life histories of *Pythium* and *Rhizoctonia* are correlated with the symptoms induced and have been studied on red pine in some detail. The account below is based on both visual and cultural examinations of damping-off foci, of over 100,000 damped-off and associated healthy seedlings in various Wisconsin forest nurseries and on studies made in the greenhouse, where seedlings were grown in naturally and artificially infected soils over a wide range of controlled soil temperature, moisture, and acidity. The details of these trials are omitted because of their volume.

PENETRATION AND PARASITISM

Pythium and *Rhizoctonia* differed in their respective growth habits in relation to the substrate. *Rhizoctonia* appeared only slightly inhibited by low soil moisture and seemed strongly aerobic. *Pythium*, on the other hand, was semiaquatic and capable of growth deep in the soil. The influence of these differences in growth habit and of host resistance on penetration was examined.

The growth of *Rhizoctonia* over the soil surface in very high humidity has been observed by various workers (e. g., C. Roth, *15*) and was found by the writers to be much more rapid than growth within the soil. However, examinations indicated that though the fungus spread rapidly by this means and mycelium frequently enveloped the several-day-old stems, it often failed to penetrate the hypocotyl. Occasionally close mycelial contact produced minute rudimentary lesions, but these did not ordinarily develop to the point of causing damping-off.

This apparent resistance of seedling hypocotyls was tested in the greenhouse with thirty-five 3- to 5-day-old seedlings that were inoculated with 4-mm. blocks of a potato agar culture of *Pythium*. A similar group was inoculated with *Rhizoctonia*. One block was placed against each seedling on a small paper platform three-eighths inch above the soil surface. The inoculation was made in a glass chamber in the greenhouse at 24° C. and approximately saturated humidity.

The results showed that *Rhizoctonia* mycelium quickly surrounded the hypocotyls, occasionally formed incipient lesions, but failed to develop further. The mycelium frequently grew down into the soil. Two days later seedlings began to damp off and continued until 93 percent were destroyed. *Pythium* attacked the seedlings directly at the point of inoculation, destroying 17 percent before it entered and attacked from the soil. Six days were required after inoculation before attack by *Pythium* through the soil became general. Eventually it killed all seedlings. Apparently the hypocotyl was highly resistant to *Rhizoctonia* and moderately resistant to *Pythium*. Root tissues remained susceptible.

Examination of seedlings in the nursery and in the greenhouse, when they were grown under controlled conditions, indicated that *Rhizoctonia*, when growing above the ground, could attack seedlings just emerging. Many seedlings around the margins of damping-off foci and with only their curved hypocotyls protruding from the ground were found damped-off. Frequently an enlarging lesion was found at the top of the curve, while parts adjacent to and under the ground were healthy. The evidence that attack was by aerial or surface mycelium confirmed similar observations by C. Roth (*15*). Apparently hypocotyls of very young seedlings were susceptible to aerial mycelium of *Rhizoctonia*, but they quickly gained resistance. The upper root and crown remained susceptible for a longer period. However, the cotyledons remained susceptible long after the hypocotyls had acquired resistance. Thus aerial *Rhizoctonia* mycelium may cause much damage through this means of entrance—and even through the needles of 2- or 3-year-old trees.

The growth of *Pythium* occurred at various depths within the soil. It appeared above the soil surface only under very humid conditions when growing over dead tissues. Its attack was largely confined to the roots. The point of infection appeared to be determined by the age of the tissues, the moisture content of the soil, and thus by the depth of the fungus in the soil. If the soil was quite wet, the infection was commonly close to the surface; if dry, the lesions appeared at deeper levels.

The longer susceptibility of seedlings to *Pythium* than to *Rhizoctonia*, reported by C. Roth (*15*), was confirmed by the writers. It appeared to result from differences in growth habit of the two fungi, correlated with tissue maturity of the host. For example, the older parts of the seedling (i.e., hypocotyl, crown, and upper root) first became resistant. *Rhizoctonia*, because it developed most profusely in the surface layers of soil or superficially over the soil surface, was early confronted by these maturing tissues, where its effectiveness as a pathogen was limited. However, *Pythium* could grow in the soil to depths equaling those normally attained by the roots of red pine seedlings during their first summer. Below the levels where *Rhizoctonia* was highly active and in a region accessible to *Pythium*, susceptible root tissue was being continuously produced. These roots were readily attacked by *Pythium*. Seedlings more than 6 weeks old showed typical root rot rather than damping-off.

MECHANICAL INJURY AND FUNGUS PENETRATION

Mechanical injury to conifer seedlings has influenced damping-off. C. Roth (*15*) and Arnold Hansson [6] found various arthropods associated with seedling hypocotyl injury at the soil surface. Wilde (*19*) reported soil nematodes in healthy seedlings as possibly important in opening infection courts for damping-off fungi. The writers observed in various Wisconsin nurseries that seedlings decapitated by birds appeared to damp off more severely than uninjured seedlings. While some seedlings were killed directly by such treatment, the survivors commonly had injuries that seemed not only to open infection courts but also to weaken the seedlings, making them more susceptible to damping-off.

The influence of two types of injury on susceptibility was studied experimentally in the greenhouse. Three hundred 3- to 7-day-old red pine seedlings were pierced at the base of the hypocotyl and root crown with approximately 35 minute holes made with insect micropins having a diameter of about 0.1 mm. Immediately after injury the seedlings in groups of 25 were transplanted into twelve 4-inch pots of Plainfield sand, 4 of which had been inoculated at their surface with *Pythium* F-111-A, 4 with *Rhizoctonia* F-5, and 4 left uninoculated as controls. Two other groups of 300 seedlings each were transplanted into 2 similar sets of 12 pots. Immediately after transplanting, the cotyledons of all seedlings in 1 group were clipped just above the terminal bud. The 300 uninjured seedlings of the third group served as controls. Incubation was at 24° C. The humidity was rather low, and consequently little damping-off of uninjured seedlings occurred. Counts were made at appropriate intervals. The losses are recorded in table 2. From this summary table it is evident that piercing alone so severely injured the seedlings that many died as a direct result of the injury. Piercing, moreover, facilitated fungus action. Losses in the *Rhizoctonia* and *Pythium* pots of pierced seedlings exceeded those in the controls by 50 and 53 percent, respectively. Clipped seedlings were much more severely attacked by *Pythium* (25 percent) than those not so injured (2 percent), but losses were not so great as in seedlings with the hypocotyl punctures (91 percent). Clipping had no discernible effect on the injury by *Rhizoctonia.* Some damping-off occurred in both clipped (6 percent) and unclipped (8 percent) seedlings. In all cases this was much less than in the pierced seedlings (94 percent). No damping-off occurred in the controls.

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[6] Correspondence.

TABLE 2.—*Influence of seedling injury on damping-off of red pine by Pythium and Rhizoctonia*

Treatment	Seedlings killed in soils, according to inoculation [1]		
	Rhizoctonia	*Pythium*	Uninoculated
	Percent	*Percent*	*Percent*
Pierced seedlings	94	91	44
Clipped seedlings	6	25	0
Uninjured seedlings	8	2	0

[1] Values based on 100 transplanted seedlings. Records were taken 2 weeks after treating and transplanting.

The above experiment had four replications of the nine treatments. Results from the different replications were consistent and showed that minute mechanical injuries to the base of the hypocotyl in the presence of the fungi greatly increased damping-off. The injury from clipping the cotyledons had no effect on susceptibility to *Rhizoctonia* but considerably increased that to *Pythium*. The results corresponded with the nursery observation that bird injury increases damping-off.

LOCATION WITHIN THE HOST

The fungi were not limited in their location within seedlings which had developed only a small amount of secondary mechanical tissue. During the early stages of disease development, their location within the host was closely correlated with the point of original infection. In general, they were distributed as shown in figure 1. During later stages, when humidity and soil moisture were favorable, they grew throughout the seedling. With further seedling maturity the fungi became more closely restricted to the point of original infection.

Whole and dissected lactophenol mounts of 3- to 10-day-old seedlings stained with cotton blue showed either fungus to be generally present in the cells of all parenchymatous tissues of the lower hypocotyl and upper root. Only rarely, however, was mycelium found in the secondary vascular elements or woody tissues. Oögonia and sporangia of *Pythium* occurred infrequently in the host tissues. However, when infected seedlings were allowed to stand in water for 12 to 48 hours, both of these structures were formed abundantly in the surrounding medium.

EXIT FROM THE HOST

The two fungi grew freely from the diseased tissue back into the soil (*6*). Their continued saprophytic development was apparently enhanced by the nutrient supply furnished by the dead seedlings.

DISSEMINATION TO THE NEW HOST

The common means of localized spread from one host to the next was direct growth through the soil. The character of this growth differed for the two fungi. *Pythium* was usually well distributed in the seedbeds and spread, though slightly, down the drills. *Rhizoctonia* commonly radiated as much as a foot or two from an original infection point, spreading both along and across rows and killing irregularly circular patches of seedlings.

For dissemination from one nursery block to another, the fungi appeared commonly to be carried in contaminated soil by instruments, water, and wind. The capacity of *Rhizoctonia* to survive in dust is discussed in a later section.

LONGEVITY AND OVERWINTERING

The overwintering of fungi causing damping-off of conifers and their longevity in the soil have been rather obscure. On other hosts—for example, on rice—*Rhizoctonia* has been observed by Palo (*11*) to live from season to season in the sclerotial stage. Buried sclerotia lost their viability after 4 to 5 months in wet soil and 6 to 7 months in dry soil. Sclerotia on the soil surface lived for 5 and 7 months, respectively, in wet and dry soils. Gratz (*5*) found that after 6 months in soil dried down at high greenhouse temperatures *Rhizoctonia* caused as severe damping-off of cabbage seedlings as occurred in the soil before drying.

The longevity of conifer damping-off fungi was studied in various field trials, the data for which have been omitted because of their volume and because the inadequate control over conditions allowed possible error. In general, the trials indicated that both *Pythium* and *Rhizoctonia* could live for more than a year.

The survival of damping-off fungi in Wisconsin has been studied under controlled conditions. The soil used was virgin Plainfield sand from a jack pine woods near Wisconsin Rapids. When a sample was seeded to red pine in the greenhouse under conditions favorable for damping-off, it was found entirely free from *Pythium* and *Rhizoctonia*. Two lots of soil were inoculated with pure whole-oat cultures of *Pythium* and *Rhizoctonia*, respectively. After the fungi had become established, the soil was passed through sterilized screens to remove the oats, placed in sterilized glazed jars, and seeded to red pine. Throughout the experiment, which was begun in the fall, every precaution was taken to avoid contamination; i. e., the soil was handled on a sterilized table with sterile instruments, the jars were tightly covered with nonmoistureproof cellophane, and all watering was with distilled water. Severe damping-off occurred in all jars. Isolations from damped-off seedlings, yielding only the fungi which had been introduced, indicated that no contamination occurred. A third lot of naturally infected nursery soil was planted in the same way. It contained about equal amounts of *Pythium* and *Rhizoctonia*. After damping-off was complete, the three lots of soil were allowed to dry to 10 percent of their moisture-holding capacity.

The *Pythium* soil was placed in 240 sterile 6-ounce bottles, 160 gm. of soil per bottle. Half of these were maintained at approximately 10-percent saturation while the remaining 120 were brought to 60-percent saturation with sterile distilled water. The bottles were closed tightly with screw caps to prevent contamination and evaporation. Half of the bottles in each moisture group were packed in sand with their necks protruding and placed out of doors. The remaining bottles were stored in the laboratory. Laboratory temperatures fluctuated around 20° C. while those in the field changed with the seasons. During the winter months the soil samples in the field were frozen solid. The *Rhizoctonia* and naturally infected soils were treated similarly. The 3 lots of infected soil, each having 2 different moistures and being stored at 2 different temperatures, provided 12 different sets of bottles (table 3). There were 60 bottles in each set, or 720 in all.

Table 3.—*Damping-off values used for analysis of variance and correlation coefficients with corresponding mean values for testing significance of differences between any 2 locations, any 2 test dates, or any 2 organisms*

Organism	Moisture content	Locations [1]	Damped-off seedlings from 210 seeds planted after inoculated soil had stood for the number of weeks specified										Means	
			8	11	14	17	20	23	28	36	49	55	Locations [2]	Organisms [3]
	Pct.		*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No*		
Natural	10	Field	31	60	52	42	10	84	39	78	64	28	49	46
		Laboratory	42	31	51	43	4	25	21	35	12	14	28	
	60	Field	49	60	80	65	22	59	22	75	1	66	50	
		Laboratory	79	67	74	46	2	89	26	69	93	37	58	
Mean [4]			50	55	64	49	10	64	27	64	43	36		
Pythium	10	Field	54	81	85	70	0	70	36	5	33	55	49	47
		Laboratory	49	74	85	39	0	27	4	15	48	45	39	
	60	Field	46	26	72	62	10	96	57	40	80	86	58	
		Laboratory	23	36	95	52	1	84	41	28	72	11	44	
Mean [4]			43	54	84	56	3	69	34	22	58	49		
Rhizoctonia	10	Field	25	15	38	36	17	72	41	1	42	65	35	32
		Laboratory	36	44	69	21	32	64	19	13	25	0	32	
	60	Field	51	66	66	64	28	14	41	4	41	0	38	
		Laboratory	12	37	25	18	7	25	5	69	18	5	22	
Mean [4]			31	41	50	35	21	44	27	22	32	18		

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[1] Places of storage for containers and soil samples during the course of the experiment.
[2] Minimum significant difference (at the 5-percent level) between any 2 location means = 19.59.
[3] Minimum significant difference (at the 5-percent level) between any 2 organism means = 9.79.
[4] Minimum significant difference (at the 5-percent level) between any 2 test-date means = 30.97.

To test the activity of the fungi on each date at various intervals following inoculation seventy-eight 4-inch pots of autoclaved Plainfield sand were placed on a clean greenhouse bench. One section of a sterilized Petri dish was used under each pot to provide for subirrigation. Duplicate bottles of soil from each treatment were employed in the test and their contents used to cover the 35 seeds in each of 6 pots. There were thus 6 replicates representing each location for each moisture content of each of the 3 infection types, or 72 pots in all. Seed in the 6 remaining pots was covered with autoclaved soil and served as controls. After seeding, each pot was covered with a truncated celluloid cone, the top of which was plugged with cotton. Seedlings were counted 5, 10, and 15 days after emergence. Total damping-off and survival were recorded at each count for the 6 pots. After the third count these values were summed and from them were calculated total emergence, and preemergence and postemergence damping-off. The amounts of postemergence and preemergence damping-off, though not the same, were of the same order. Consequently, only the former were used in presenting the results. These figures for 10 different test dates from March 1939 to March 1940 are given in table 3 with the mean damping-off values calculated for the different test dates, locations, and inoculations, respectively.

Viability tests extended over the period from December 1938 to March 1940. Those made during the first 2 months showed no differences in viability or activity of the fungi and have, for convenience, been omitted from the analysis.

The statistical significance of the amount of damping-off in the three inoculation series with different moistures and with different locations was determined by analysis of variance. This analysis is given in table 4.

TABLE 4.—*Analysis of variance for the number of red pine seedlings damped-off in pots inoculated with soil samples containing Pythium, Rhizoctonia, and naturally contaminated soil* [1]

Variation due to—	Degrees of freedom	Sum of squares	Mean square	*F* [2]
Organisms	2	6,006.46	3,003.23	6.24**
Moistures	1	1,197.01	1,197.01	2.49
Locations	1	2,475.21	2,475.21	5.15*
Test dates	9	25,959.74	2,884.42	6.00**
Twentieth week vs. others	1	12,546.07	12,546.07	26.09**
Between others	8	13,413.67	1,676.71	3.49**
Organisms × moistures	2	1,950.87	975.43	2.03
Organisms × locations	2	145.87	72.93	.15
Organisms × dates	18	9,191.54	510.64	1.06
Moistures × locations	1	161.00	161.00	.33
Moistures × dates	9	1,483.41	164.82	.34
Locations × dates	9	3,669.21	407.69	.85
Error	65	31,261.67	480.95	
Totals	119	83,501.99		

[1] The various samples had been exposed to different environments for periods ranging from 8 to 55 weeks.

[2] * = Significant at the 5-percent level; ** = significant at the 1-percent level.

Since the mean square corresponding to differences between organisms exceeded the error mean square to an extent statistically significant at the 1-percent level the existence of real organism differences as regards damping-off may be inferred with small risk—the probability of differences between organisms as large as or larger than those observed occurring through chance fluctuations alone being less than 1 in 100.

Statistically significant differences were found also between locations (at the 5-percent level) and between test dates (at the 1-percent level). One conspicuous feature in table 3 is the fact that the damping-off values for the twentieth week are considerably out of line with the values obtained in the other weeks. Therefore, in table 4 the variation due to the comparison of the twentieth week with the others has been isolated, and it may be noted that the sum of squares for this single degree of freedom is nearly as large as the sum of squares for the 8 degrees of freedom corresponding to the eight independent comparisons between the other nine test dates. The mean square corresponding to the comparison among the other test dates is significant at the 1-percent level, showing the existence of differences between test dates as regards damping-off even when the anomalous data for the twentieth week is disregarded. No statistical significant differences between moistures were detected, nor were any of the first-order interactions statistically significant. This latter finding implies that such differences as existed were consistent throughout; e. g., differences between organisms were independent of location.

The nature of the differences for those factors showing statistical significance may be determined by comparing the differences of means given in table 3 with the corresponding 5-percent minimum significant differences included as footnotes to table 3.

Thus it is apparent that losses from *Rhizoctonia* soil were significantly less than from *Pythium* soil or from naturally infected soil.

The fungi were significantly more active when stored in the field than in the laboratory, except *Rhizoctonia* at 10-percent moisture and those in natural soil at 60-percent moisture.

Discarding the anomalous data for the twentieth week, linear regressions of damping-off means on test dates were calculated for the respective organisms, and the regression coefficients obtained were −.3334, −.2775, and −.4092 for naturally infected and *Pythium*- and *Rhizoctonia*-inoculated soils, respectively. The regression coefficients do not differ from zero to a statistically significant extent when considered alone, but they are statistically significant at the 5-percent level when considered collectively, suggesting that there may be a tendency for activity to decrease. With *Pythium* the deviations from linearity, significant at the 5-percent level, suggest that the decline in activity, if existent, is not linear.

Nevertheless, damping-off fungi survived well over a year. Differences in soil moisture did not influence longevity during the time studied. Considering such longevity in the soil, the wide distribution of damping-off fungi and the prolonged infection of nursery soils is not surprising.

OCCURRENCE AND DISTRIBUTION

The distribution of damping-off fungi seems important because of (1) its bearing on nursery establishment and practice, (2) its possible relation to the natural distribution (*21*) and reproduction of pine species, and (3) the relation these species may have to root rot after transplanting.

Damping-off fungi have apparently been long established in Wisconsin since the disease had appeared by 1913 at Trout Lake (*8*). A 25-percent loss occurred in 1933 in the first seeding at the Griffith State Nursery on land reclaimed from an abandoned field. The first crop at the Gordon State Nursery on land where heavy sod was broken also was severely attacked.

The occurrence of the fungi in Wisconsin has been determined by the damping-off induced and by subsequent isolations from damped-off seedlings. Numerous isolations were made during the summers of 1937, 1938, and 1939 from seedlings damped-off in 6 nurseries in northern and central Wisconsin and in several nonnursery sites. Before plating, ¼-inch sections of hypocotyl, including both incipient infection and healthy tissue, were washed in clear water with a camel's-hair brush, then rinsed vigorously in 2 changes of sterile water, and placed on Petri plates of water agar. Damping-off fungi were readily differentiated after a few days.

The results are first considered in connection with the reaction of the soil from the locations examined.

THE INFLUENCE OF ACIDITY

Soil reaction was studied in relation to the occurrence of damping-off in sandy soils. In table 5 the isolation results, arranged according to acidity, show that in soils more alkaline than pH 5.85 *Pythium* was isolated more frequently than *Rhizoctonia*. The samples for the pH readings were taken from the top inch of soil. In soils more acid than pH 5.85, however, *Rhizoctonia* was almost always more common. Higher *Pythium* damping-off was correlated with acidities less than pH 5.85, giving a coefficient of —.6035. This value is highly significant, according to Fisher's table of *r*, where, with 20 degrees of freedom, a coefficient of —.413 is required for significance at the 5-percent point. The figures in table 5, representing partial percentage values of the same total, show that *Rhizoctonia* was correlated with acidities greater than pH 5.85. Although the division point between the 2 fungi seems quite sharp at pH 5.85, this point may doubtless be raised or lowered somewhat under other conditions, especially those of weather.

The results indicated that with the soils studied *Pythium* was favored by neutral or slightly acid soils while *Rhizoctonia* predominated on the very acid soils. This relationship has been confirmed in greenhouse and nursery studies (*16, 17*). It is noteworthy that occurrence of the disease due to 1 fungus or the other was not restricted by soil reaction over the range examined.

THE INFLUENCE OF WEATHER

Weather often has appeared to influence the occurrence of damping-off and probably the distribution of the fungi as well. This subject is so important that it is given detailed consideration elsewhere (*16, 17*), and attention is directed here to the influence of different kinds of soil.

TABLE 5.—*Distribution of Pythium and Rhizoctonia in Wisconsin forest nurseries* [1]

Location near—	Soil		Seedlings plated	Platings positive for *Pythium* and *Rhizoctonia*	Yield of positive platings			
	Type	Reaction [2]			*Pythium*		*Rhizoctonia*	
		pH	*Number*	*Number*	*Number*	*Percent*	*Number*	*Percent*
Rhinelander	Vilas sand	[3] 4.98	8	[4] 1	0	0	1	100
Port Edwards	Plainfield sand	[5] 4.98	56	54	0	0	54	100
Do	do	[5] 5.15	82	81	15	18	66	81
Gordon	do	[5] 5.16	13	7	0	0	7	100
Madison	Silt loam	5.26	17	15	3	20	12	80
Trout Lake [6]	Vilas sand	5.35	19	10	8	80	2	20
Wisconsin Rapids	Plainfield sand	5.39	16	16	0	0	16	100
Hayward	do	[5] 5.43	6	2	0	0	2	100
Trout Lake [7]	Vilas sand	5.53	25	18	9	50	9	50
Port Edwards	Plainfield sand	5.75	5	5	0	0	5	100
Do	do	5.82	57	52	0	0	52	100
Do	do	5.84	30	30	7	23	23	77
Do	do	5.84	17	17	0	0	17	100
Do	do	5.84	16	16	3	19	13	81
Rhinelander	Vilas sand	5.88	7	4	3	75	1	25
Hayward	Plainfield sand	5.93	13	11	11	100	0	0
Gordon	do	6.08	40	15	15	100	0	0
Trout Lake	Vilas sand	6.22	17	15	13	87	2	13
Do	do	6.42	51	51	51	100	0	0
Port Edwards	Plainfield sand	6.55	22	22	6	27	16	73
Trout Lake	Vilas sand	6.55	28	19	19	100	0	0

[1] The table is arranged according to soil reaction progressing from high to low acidity.

[2] Determinations were made with a quinhydrone electrode and checked with a glass electrode.

[3] Spring-seeded red pine treated at seeding with 2-percent sulphuric acid to control damping-off.

[4] Plates not giving either *Pythium* or *Rhizoctonia* were either negative or showed saprophytes or dubious parasites.

[5] Fall-seeded red pine treated at seeding with 2-percent sulphuric acid to control damping-off.

[6] Woods soil not in nursery.

[7] Field soil not in nursery.

THE INFLUENCE OF SOIL TYPE

The relative amounts of damping-off were studied in abandoned field soils and in soils with forest cover. Five 4- by 4-foot seed beds were planted and protected from birds and rodents in the spring of 1938 on Plainfield sand at each of three sites. These were: (1) A nursery block used for experimental studies during 1937 and inoculated at that time with damping-off fungi, (2) a field abandoned for 11 years, and (3) a small opening in a middle-aged stand of jack pine with an oak brush understory. The two latter locations were 5 rods apart and differed only in the type of cover and such factors as light and moisture arising from the cover differences. Undoubtedly, the microbiology of these soils also differed. In the nursery the soil contained a moderate amount of organic matter and had a reaction of pH 5.5. In the field soil organic matter was low, the pH was 5.4, and the vegetation was a sparse stand of weeds and bunchgrass. In the woods organic matter was relatively high, the pH 5.8, and the conspicuous ground cover lowbush blueberry and bunchgrass. The beds were spaded thoroughly and half of each was inoculated with one-third quart of a whole-oat culture of *Pythium* and a similar amount of *Rhizoctonia*. The beds were seeded to red pine, and after emergence a record was kept of the number of seedlings damped-off and surviving in all plots. At about 30 days after the seedlings had attained relative immunity, all the tiny trees were pulled and observations made on their development. This procedure was followed three successive times. No additional inoculation was employed in the second and third seedings. Except for a diminution of the effect of inoculation, the results of the later seedings were in agreement with those of the first.

Damping-off results from the five replications of the first seeding are given only in summary form. In the uninoculated plots in nursery, field, and woods, 9, 15, and 1 percent, respectively, of the emerged seedlings damped off, whereas in the inoculated plots on the same sites the losses were 33, 25, and 14 percent, respectively. All attempts failed to isolate either *Pythium* or *Rhizoctonia* from seedlings damped-off in the uninoculated woods plots while both fungi were isolated with ease from all other situations. The 14-percent loss in inoculated plots in the woods and the 1-percent loss from the uninoculated plots suggested that damping-off fungi were very scarce, if present at all, in the woods before inoculation. Damping-off declined in the inoculated woods plots with subsequent seeding. In the spring seeding of 1939, though a very small amount of damping-off occurred in the inoculated plots of the preceding year, it was no longer possible to isolate *Pythium* or *Rhizoctonia* from the seedlings. Damping-off fungi seemed unable to compete successfully with the organisms already in the woods soil. These results accorded with experience at the Griffith State Nursery, where damping-off was invariably much worse on old field soil than in newly cleared forest soil. Thus it appeared that damping-off fungi may be practically or entirely absent from certain Wisconsin forest soils, but that in abandoned field soils of the same type they may build up to levels as great as those found in contaminated nurseries.

A somewhat similar experiment to determine the influence of site on occurrence of the fungi and disease was conducted in the summer of 1939 at the University of Wisconsin arboretum. The following three locations were chosen: (1) An eroded and exposed sandy knoll in an abandoned orchard having heavy bluegrass sod and a soil reaction of pH 5.4, (2) another sandy knoll with a middle-aged stand of black locust and oak and a pH of 5.3, and (3) a plot covered by a pure stand of oak and having a heavy mull soil with a strongly acid reaction of pH 4.2.

Twelve circular wire covers 15 inches in diameter were placed on each site to keep out birds and mice (fig. 2). In order that conditions of light and soil moisture might be more nearly comparable on all sites, a cheesecloth cover was attached to the square of metal screen covering those beds located in the exposed orchard site. Among the 12 beds on each site were randomized 4 replications of each of the following treatments: (1) one-sixth pint of a corn meal-sand culture of *Pythium* and an equal amount of *Rhizoctonia* mixed into the upper 2 inches of soil at the time of seeding; (2) one-third pint of a sterile corn meal-sand mixture introduced into the upper 2 inches of soil; and (3) no treatment. Two hundred red pine seeds were spread over the smoothed surface of each bed and covered with one-fourth inch of autoclaved potting sand. Damped-off and surviving seedlings were counted and recorded at appropriate intervals after emergence. Damped-off seedlings were plated and, where possible, the causal fungi determined as given in table 6. The plots were seeded and results taken three successive times, the only significant difference in result being a small decrease in damping-off in the second and third seedings of the inoculated plots. The results of the first seeding are given in table 6.

FIGURE 2.—A representative unit of field plots employed in studying the occurrence of damping-off in various natural environments. Each covered plot was sprinkled with white sand just before photographing.

TABLE 6.—*Damping-off of red pine on 3 natural sites in the arboretum, Madison, Wis., and the respective importance of the 2 fungi causing the loss*

Site	Treatment	Seedlings counted				Fungi isolated			
		Germinated		Damped-off		*Pythium*		*Rhizoctonia*	
		Number	*Percent*[1]	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
Sandy, exposed, pH 5.4.	Inoculated corn meal and sand	216	29	42	19	2	50	2	50
	Sterile corn meal and sand	485	66	29	6	(2)	------	(2)	------
	No treatment	568	78	17	3	(2)	------	(2)	------
Sandy, locust cover, pH 5.3.	Inoculated corn meal and sand	161	22	82	51	2	15	11	85
	Sterile corn meal and sand	514	70	84	16	2	33	4	67
	No treatment	522	71	54	10	1	11	8	89
Mull, oakcover, pH 4.2.	Inoculated corn meal and sand	83	11	49	59	12	60	8	40
	Sterile corn meal and sand	349	48	33	9	8	80	2	20
	No treatment	402	56	66	16	9	82	2	18

[1] These figures are based upon 728 viable seeds as determined by germination tests.

[2] All platings made of the small number of seedlings damped-off in the plots treated with sterile corn meal and sand and the untreated plots on the sandy site were negative for *Pythium* and *Rhizoctonia*.

Some damping-off occurred in the uninoculated beds on all sites. Inoculation, however, in all cases greatly increased the amount. Corn meal and sand alone appeared not significantly to influence the amount of damping-off. Mean losses in the uninoculated beds (sterile corn meal and sand and no treatment) for the three locations, sandy exposed, sandy woods, and mull woods, respectively, were 4.4, 13.3, and 13.2 percent. Comparable losses for the inoculated beds were 19.4, 51.0, and 59.0. The losses on sandy and mull soils in the woods were approximately equal for the inoculated and uninoculated beds and suggested that damping-off fungi occur naturally in both types of soil. The fact that damping-off on the exposed site was much less severe than in the woods was probably due to less favorable humidity and soil moisture rather than to other differences caused by the shade. Germination was retarded because of dry soil, and after emergence moisture was inadequate until the seedlings had passed the stage of practical susceptibility. These results indicate that in southern Wisconsin damping-off fungi may be equally abundant in both light and heavy, deciduous-wooded soils.

The differences of soil type largely determined which of the two important damping-off fungi would predominate (table 6). In the heavy soil of untreated plots *Pythium* predominated, causing 82 percent of the total loss, while in the sandy soil *Rhizoctonia* was more abundant, causing 89 percent of the loss. These results accorded with Hartley's observation (*7*) that soils high in humus favored *Pythium* while *Rhizoctonia* was more abundant in soils low in humus.

In heavy acid soils the results accorded with those of Gäumann (*4*) and Buchholtz (*1*) obtained with seedlings on similar soils. However, in controlled studies with Plainfield sand (table 5) *Pythium* responded quite differently to soil reaction. This subject is considered further elsewhere (*16*).

DISCUSSION

Variations in the success of control measures applied in Wisconsin against damping-off among forest seedlings and, more particularly, the desire to eliminate empirical drastic treatments that ruin soil fertility have called for more information concerning the disease. The causal fungi have been identified as *Pythium* and *Rhizoctonia*—two organisms that are active not only elsewhere in the United States (*7*) but also in Europe (*15*). Other organisms reported to cause damping-off have been found either not at all or so infrequently as not to be significant.

Since there are two causal agents, the damping-off problem is most clearly defined as dealing with two separate diseases. The dual nature of the situation appears in several respects. The symptoms caused by these two fungi, respectively, when operating alone, are distinct and seem correlated with their growth habits. Likewise, in spite of the striking similarity of the two causal agents, certain observed variations in their mode of attack, dissemination, and longevity are conspicuous. Their distribution in Wisconsin is widespread, but the predominance of one over the other is clearly influenced both by weather and by soil type and acidity.

For an improved understanding of the situation, a detailed examination of the influence of environment on host, parasite, and disease was requisite. The results of such studies are reported elsewhere (*16, 17*).

SUMMARY

Pythium irregulare and *Rhizoctonia solani* have been found the most destructive of the fungi that cause damping-off of *Pinus resinosa* in Wisconsin forest nurseries. Though species of *Fusarium* were isolated, they occurred in conjunction with the other fungi and in inoculation tests were only slightly, or not at all pathogenic.

Differential symptoms were induced by *Pythium* and *Rhizoctonia*, respectively, when each was free from complicating mixtures with other organisms. The symptoms described appeared correlated with the respective growth habits of the two fungi. *Pythium* attack occurred at various depths in the soil and was determined by root maturity and the location of the fungus. For the most part, *Rhizoctonia* attack was confined to the upper one-half inch of soil and to saturated air immediately above the soil.

Seedlings with elongating hypocotyls were subject throughout to attack by aerial mycelium of *Rhizoctonia.* After elongation had ceased, however, it appeared that the cotyledons and the primary shoot were the susceptible parts above ground.

Seedling injury, within limits, increased damage from damping-off.

The life history in relation to pathogenesis of these two fungi seemed relatively simple. Both lived in the soil and invaded injured seedlings more easily than uninjured seedlings. They grew through the cortical tissue of living seedlings, all through dead tissue, and out into the soil again. Distribution locally was by growth through the soil, and at a distance, by means of contaminated soil or other material. *Rhizoctonia* survived well in soil containing only 10 percent of moisture and dry enough to blow as dust.

Both *Pythium* and *Rhizoctonia* were capable of surviving more than a year in sandy soil.

In six Wisconsin nurseries and several nonnursery sites examined, one fungus or both were commonly found. Their occurrence was influenced by soil reaction, weather, soil type, and ground cover. Plainfield sand with jack pine (*Pinus banksiana*) or jack oak (*Quercus ellipsoidalis*) cover was associated with the presence of little or none of the damping-off.

The apparently strong influence of acidity, temperature, and moisture called for a detailed study of these factors in relation to damping-off.

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SOLAR RADIATION AND FOREST FUEL MOISTURE[1]

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INTRODUCTION

A major contribution to progress in forest fire prevention and control during the past 10 years has been the development and widespread application of methods of rating forest fire danger.[2] Fire danger rating systems are now in use in all the forest regions of the United States. They have been described by Gisborne (*8*, *9*),[3] Brown and Davis (*2*), Curry et al. (*4*), Matthews (*13*), Jemison,[4] and others. Under each of these systems the major factors affecting fire danger are measured and the measurements are integrated by means of charts, tables, or some mechanical device into ratings, on a numerical scale, which are free from the serious errors common in estimates of fire danger based on personal judgment alone. The numerical ratings are usually defined in terms of probable fire behavior or of manpower required for suppression. They serve as a guide to efficient distribution of fire-control funds and personnel.

All these fire-danger rating systems include measurement of wind velocity and of fuel moisture content. Wind velocity, an extremely important factor whenever fuels are dry enough to burn, is usually measured with standard instruments. Fuel moisture content is more difficult to determine, because of the complex nature of fuels. Lightweight materials such as fallen leaves, needles, and twigs and dead grass respond readily to changes in atmospheric conditions. The condition of this litter[5] is determined indirectly from measurements of air temperature and humidity or, more commonly, is determined directly by means of calibrated "wood sticks," the moisture content of which changes in harmony with that of natural fuels similarly exposed. The moisture content of heavy fuels, such as large branches, logs of all sizes, snags, and deep, buried layers of duff, changes slowly, and its measurement requires different techniques. In some regions it goes through a seasonal cycle, so that calendar date is a good index of cumulative drying. Elsewhere, elapsed time since rains of different amounts serves as an index. The seasonal cycle differs between north- and south-facing slopes and between steep and gentle slopes, according to the

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[2] "Forest fire danger" as used here, is a general term signifying the combination of variable elements that determines whether fires will start, and if so, the probable rate of spread and extent of damage.

[3] Italic numbers in parentheses refer to Literature Cited, p. 175

[4] Jemison, G. M., the measurement of forest fire danger in the eastern united states and its application in fire prevention and control. Appalachian Forest Exp. Sta. Tech. Note 50, 59 pp., illus. 1942. [Processed.]

[5] Litter is the uppermost layer of organic materials in the forest floor, the structure of which has not been materially altered by decomposition.

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position of the sun, hence, the effectiveness of radiation. Another important element in fuel moisture conditions is stage of development of forest vegetation—the living fuel. This is ordinarily gaged by calendar date, ocular estimate, or actual measurement in the laboratory.

Determination of the best methods for measuring the moisture content of all types of fuels requires a knowledge of the interplay of controlling factors. For example, standards of how, where, and when to measure litter moisture and heavy fuel moisture cannot be established unless the controls of rates of drying and moisture equilibria are understood.[6] The weight that each fire-danger element should carry in the final rating, also, can be determined accurately only when the interactions of all controlling factors are known. The significance of season, elapsed time since rain, and similar indirect indices of fuel moisture depends on how the complex fuels of a given region react to the combined effect of the atmospheric factors. Furthermore, knowledge of the interaction of controls is needed in applying the ratings. In order to utilize efficiently the manpower available for fire suppression, a dispatcher needs to know how to adjust the rating available for his locality to allow for variations in temperature, humidity, and radiation brought about by variations in cover or topography in other parts of his district not served by a danger station. A fire boss on the fire line needs to be able to adapt danger ratings based on measurements taken elsewhere to fuel and weather conditions for areas in the path of the fire.

Although considerable effort has been made in the past to isolate the influence of each weather element, very little has been done to determine the effect of solar radiation on rates of drying of fuels and on the moisture equilibria of these fuels. The influence of shade on fuel and micro-climate has been discussed frequently. Stickel (*15*) obtained data confirming the importance of a timber canopy in maintaining high fuel moistures. He also found that in the mixed-hardwood-softwood forest region of the west-central Adirondacks, evaporation, hours since rainfall, duff temperature, air temperature, depression of the dew point, and relative humidity are most closely associated with litter moisture, in the order given. Mitchell (*14*) rated the general influence of individual weather elements on fuel moisture in the Lake States and showed how forest cover modifies atmospheric factors and hence fuel moisture. Jemison (*12*) found that absolute atmospheric moisture alone explained about as much of the variation in litter moisture content as did 15 weather factors combined. The work of these and other investigators brought out only indirect evidence, however, of the importance of solar radiation as a control of fuel moisture.

Gast and Stickel (*6*) were probably the first to investigate specifically the effect of sunlight on the rate of drying of forest fuels and consequently on fire danger. They compared fuel moistures in the open with those under the shade of bobbinet screens and found that the reduced radiation resulted in slower drying rates. From these investigations they concluded that degree of cloudiness could be used to gage fire danger and also that the amount of shade-producing cover on a cut-over area is an important criterion of the relative danger (dryness) on the area.

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[6] Equilibrium moisture content may be defined as the asymptotic value which the actual moisture content approaches if the litter is subjected to a given temperature and humidity for an infinite length of time.

Hayes (*11*) measured air temperature, relative humidity, wind, litter moisture, maximum litter temperature, precipitation, and soil moisture on north and south slopes at each of three elevations in northern Idaho, and made a comparison of conditions as regards the first six of these elements on north slopes with those on south slopes. His results showed significant differences between the two aspects for all six elements except precipitation. He pointed out the importance of insolation as a control of fuel moisture, as indicated by measurements of maximum litter temperature. On south slopes, he found minimum litter moisture sometimes varied less than ±1 percent from one clear day to the next, although maximum air temperature might range from 70° to 95° F., and minimum relative humidity from 30 to 15 percent. Hayes found that beds of pine duff on south slopes had a minimum moisture content, for a median day in August, about 2 percent lower than that of comparable beds on north slopes. His data indicate appreciable differences in fire behavior indices on north and south slopes.

The importance of solar radiation as controlling fuel moisture was mentioned by Byram (*3*). Radiation caused sticks exposed in calm air to dry rapidly. On wood sticks exposed to full sunlight, the drying action of wind was more than offset by its cooling action. Gisborne (*7*) found that sticks lying on litter were drier than those supported 10 inches above the litter surface, where circulation of air was greater.

An investigation of the influence of solar radiation on the moisture equilibria and rates of drying of forest fuels was begun by the Appalachian Forest Experiment Station in 1938, at the Bent Creek Experimental Forest, on the Pisgah National Forest, near Asheville, N. C. The investigation has included study of the effectiveness of radiation as a control of fuel moisture during different hours of the day and seasons of the year, on slopes of varied steepness and aspect. Theoretical relations between solar radiation and fuel moisture equilibria have been established and have been checked by field and laboratory experiments.

Under natural conditions, of course, fuel moisture is seldom in a state of equilibrium, but is usually increasing or decreasing. However, fuels with large exposed surfaces, such as hardwood leaf litter, approach the equilibrium moisture content after rain rather quickly. The rate of loss of moisture from forest litter in the southern Appalachian region on sunny days is so rapid that this layer of fuels may be considered as being in the equilibrium state most of the time.

The following technical discussion is intended to enable fire specialists to understand better the importance of radiation as a control of fuel moisture, and indicates work yet to be done on the problem. Suggestions are offered for use of the data, which may lead to refinements in some fire-control practices.

EXPERIMENTAL APPARATUS AND METHODS

In order to supply necessary constants in equations derived in this study, as well as to establish the effect of radiation on fuel moisture under a variety of topographic and atmospheric conditions, an "artificial sun" was constructed. This apparatus is actually a weather synthesizer, simulating variations of sunshine and wind, and making it possible to obtain quickly the effect of radiation as modified by topography on fuel moisture equilibria. It renders unnecessary the impractical alternative of measuring directly in the field the response of fuel moisture to numerous combinations of weather elements on several kinds of slope throughout a year.

Two such artificial suns are shown in figure 1. The walls of the artificial sun enclose a rectangular volume divided by a horizontal glass plate into upper and lower compartments. The lower and shallower compartment, open at both ends and at the bottom, serves as a wind tunnel. At the top of the larger compartment, twelve 150-watt tungsten bulbs are placed on the perimeter of a circle to provide radiation. It has been shown mathematically that a circular source of light or radiation gives uniform distribution of energy on a surface parallel to the plane of the source and situated at a distance from that plane equal to the radius of the circle. As the radius in this case was 9 inches, the bulbs were so placed that they were separated by a space of 9 inches from the leaf litter or other fuel tested. The whole interior of the apparatus was painted white, with the result that energy was uniformly distributed on the fuel even when only two or three bulbs were burning. The uniformity of radiation at the fuel surface was verified by careful checks with a photronic cell. The apparatus was placed directly over the natural litter in the forest or over fuel samples in the laboratory. Electric fans were used to draw air through the wind tunnel and over the fuel. Wind velocity was varied by adjusting the number of fans in operation or the openings in the box immediately behind them. Intensity of radiation was varied by turning on or off some of the bulbs; three or four bulbs, evenly spaced in the circle, were turned on or off at a time.

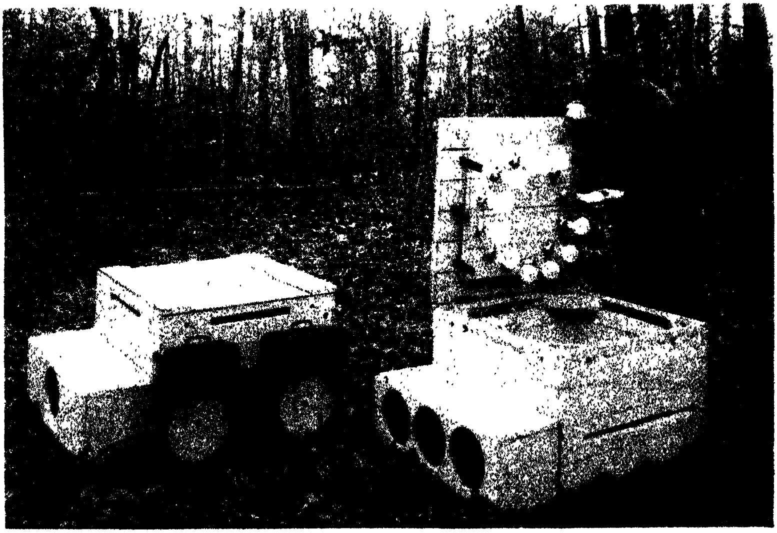

FIGURE 1.—Two artificial suns, or weather synthesizers.

Usually two of the artificial-sun devices were operated simultaneously, so that results could be checked, or one of the three factors wind, radiation, and precipitation (simulated) could be varied while all other factors were held constant. Wet- and dry-bulb temperatures were taken outside the apparatus, 6 to 8 inches above the ground, with a hand-aspirated psychrometer. Within the artificial sun, surface fuel temperature was measured by means of a recording thermometer, the ⅛-inch cylindrical bulb of which was placed on the litter and covered with a single thickness of oak leaves. Leaf samples were periodically collected, weighed, and oven-dried, and the moisture content computed on the basis of oven-dry weight. Tests varied in length from 2 hours to 2 days, according to the time required for the moisture content of the fuels to reach equilibrium.

Intensity of radiation was measured with the ice calorimeter (fig. 2), a device composed of two identical ice-containing units, each unit consisting of a thin-walled copper cup blackened inside and heavily insulated. Each insulated lid has a 2-inch hole at its center. One hole is uncovered, to admit radiation; an insulated shade excludes radiation from the other unit. Both units are exposed to the light source in the enclosed chamber of the artificial-sun apparatus. In use, each cup is two-thirds filled with ice. The difference in the number of grams of water draining from the two in a given time interval, multiplied by the heat of fusion of ice, gives the total radiant energy in calories admitted by the opening in the calorimeter lid during this interval. This energy, expressed in calories per square centimeter per minute, closely approximates the energy of solar radiation. Ten of the 150-watt bulbs gave 1.53 calories per square centimeter per minute, equivalent to very bright sunlight.

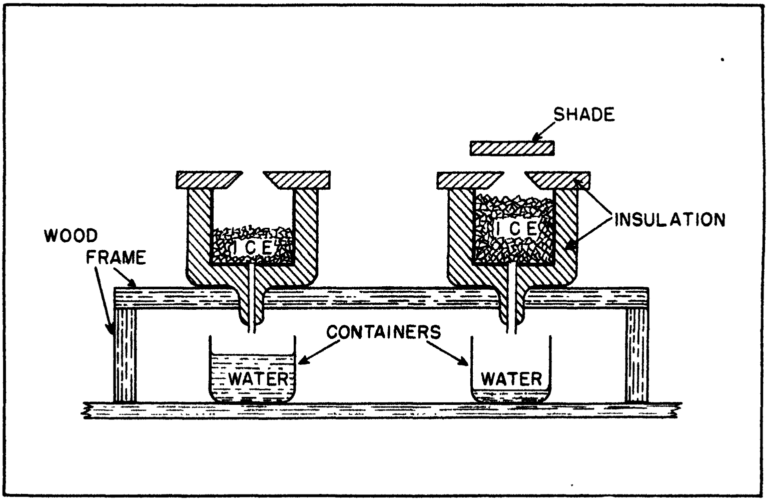

Figure 2.—Ice calorimeters used to measure intensity of radiation.

INTENSITY OF SOLAR RADIATION

The intensity of sunlight, varying with time of day, time of year, slope, aspect, latitude, and haziness of the atmosphere, can conveniently be expressed as a fraction of the theoretical maximum intensity or that received by a horizontal plane at the top of the atmosphere when the sun is at the zenith.

INTENSITY AS DETERMINED BY POSITION OF SUN, ASPECT, AND SLOPE

If atmospheric absorption is ignored, the intensity of solar radiation on a horizontal surface can be computed from the geometric position of the sun with respect to the surface. The sky may be regarded as a hemisphere, the lower boundary of which is the plane of the horizon.

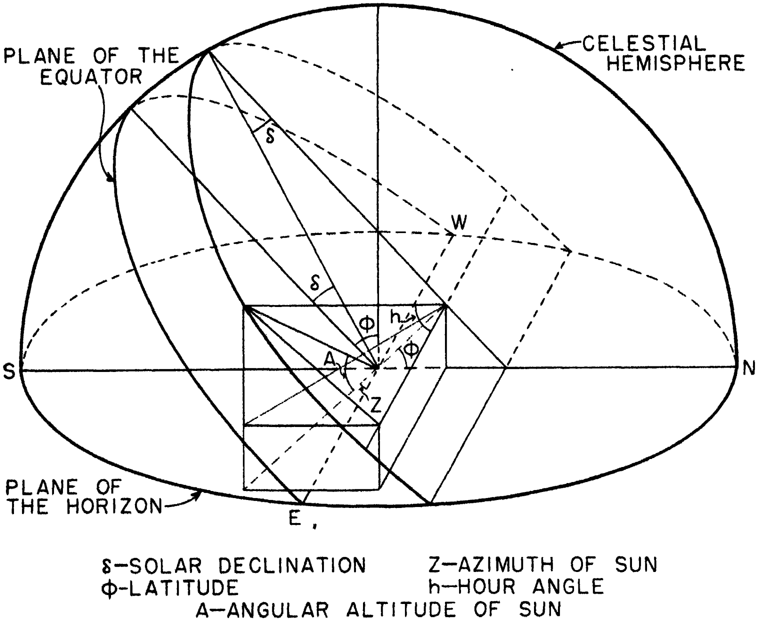

FIGURE 3.—Diagram of celestial hemisphere.

The daily paths of the sun are arcs on this hemisphere, as shown in figure 3. The angular altitude A of the sun is given by the equation

$$\sin A = \cos \delta \sin h \cos \phi + \sin \phi \sin \delta \qquad (1)$$

where δ is the solar declination, h the hour angle[7] (measured from 6 a. m.), and ϕ the latitude. The azimuth Z of the sun's position is given by the equation

$$\cos Z = \frac{\cos \delta \cos h}{\cos A} \qquad (2)$$

Figure 4 shows diagrammatically the conditions that exist when sunlight falls on a plane (mountain slope) tilted at some angle α from the horizontal and rotated clockwise through an angle β with respect to the east. The direction of the sun is along the line BC in the vertical plane CEB, and the lines BD and BE are the intersections of this plane with the tilted plane DHG and the horizontal plane EFG,

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[7] In the standard equations given in a solar ephemeris or in most civil engineering handbooks or texts such as Breed and Hosmer (*1*), from which equations (1) and (2) can be derived, the hour angle is measured from noon and the azimuth angle is measured clockwise from north. However, in this study it was more convenient to measure the hour angle from 6 a. m. and the azimuth angle clockwise from east.

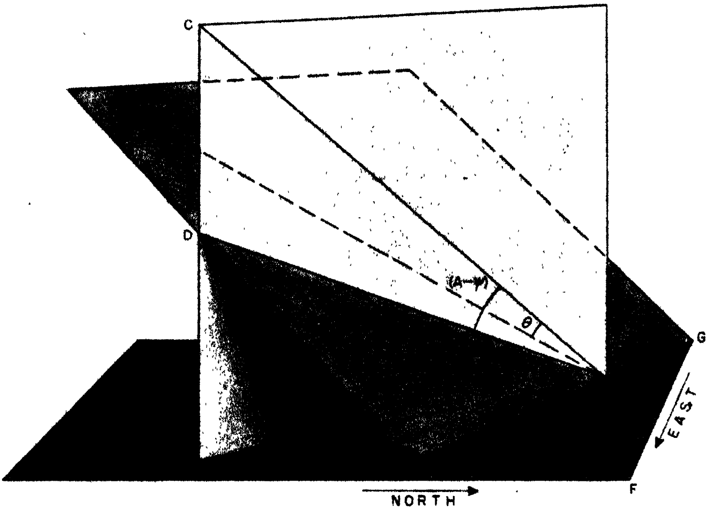

FIGURE 4.—Diagram from which the theoretical intensity of solar radiation on a tilted plane may be determined.

respectively. The angle *FGH* is the aspect angle β. For a mountain slope facing northeast, the aspect angle would be 45°; for one facing east, 90°; north, 0°; southwest, 225°. The intensity of radiation on the plane *DHG* is proportional to sin θ, which is given by the equation

$$\sin \theta = \sin (A - \psi) \frac{\cos \alpha}{\cos \psi} \qquad (3)$$

the angle θ is the angle between the direction of the sun *BC* from a given point *B* in this plane and the projection of *BC* on the plane. Angle ψ is the angle between the projection of *BC* on the horizontal plane and the line *DB* formed by the intersection of the vertical plane and the tilted plane; tan ψ is given by the equation

$$\tan \psi = \sin (Z - \beta) \tan \alpha$$

which, when combined with equation (3), gives

$$\sin \theta = \sin A \cos \alpha - \cos A \sin \alpha (\sin Z \cos \beta - \cos Z \sin \beta) \qquad (4)$$

EFFECTIVE RADIATION

The total amount of solar heat actually reaching the earth's surface is affected by atmospheric absorption of radiation. According to the law of absorption and Lambert's cosine law, the effective radiation received on any slope can be found from the equation

$$I = I_0 \sin \theta \, p^{\frac{1}{\sin A}} \qquad (5)$$

where I is the incident intensity of radiation on a tilted plane at the surface of the earth, I_0 is the intensity of radiation at normal incidence on a horizontal surface at the top of the atmosphere, and p is the fraction of radiation transmitted at normal incidence through the atmosphere. The quantity I_0, the solar constant, equals 1.94 calories per square centimeter per minute according to Forsythe (*5*). However, it is often more convenient to express I_0 as unity so that I can be expressed as a fraction of I_0.

The absorption factor p is not constant from place to place or from day to day in a given locality, because it is affected by a large number of conditions such as water vapor, smoke, and dust in the air, and by altitude. In this study p was given an estimated value of 0.7, a reasonable average for a thin layer of rather dense haze, which is common at elevations of 2,000 feet in the southern Appalachian Mountains during the fall and spring fire seasons. In western forest regions of the United States the values of p may average somewhat higher than 0.7, although it is doubtful that they can ever be much above 0.8 except at great elevations. As defined and used in equation (5), p is independent of the vertical distribution of absorbing haze particles and the angular altitude of the sun.

The quantity I/I_0, which expresses the fraction of total possible radiation that actually reaches the earth's surface as determined from equation (5), has been presented in figure 5 as a function of time of day for typical combinations of slope, aspect, and season in the southern Appalachians. (The hour of maximum radiation intensity is that when the sun's rays approach the nearest to a 90° angle with the plane of the slope.) Curves showing fractions of maximum radiation received on 20-, 40-, and 100-percent south, north, and east slopes are plotted for June 21 and December 21, dates when the sun reaches its greatest and least angular altitudes. For the calculations represented in determining sin A, a latitude of 35°30′ was assumed. Solar declinations were obtained from a solar ephemeris, northern and southern declinations having the customary positive and negative signs, respectively.

The differences in radiation on north and south slopes brought out by figure 5 are particularly significant because direct sunlight is the greatest factor in drying exposed forest fuels. In deciduous forests or on clear-cut conifer forest areas in the eastern mountain regions, fuels are unshaded, or practically unshaded, during several months of the year. The hardwood stands are leafless from November into May, or even longer at some elevations and latitudes, and this period includes the major part of the annual fire season. On clear-cut or severely burned forest areas in the West, fuels are completely exposed to the sun throughout the fire season. Obviously, if ground fuels are partially shaded by a forest canopy, radiation intensity as represented in figure 5 is modified.

Among the southern exposures, in summer the 20-percent slope receives the greatest radiation, because this slope forms an angle of almost 90° with the sun's rays at noon. The 100-percent slope is too steep to receive maximum radiation at this hour. In winter, however, when the sun is low, the 100-percent slope receives more radiation than either the 20- or the 40-percent slope. Curves for other seasons of the year would fall between those shown for June 21 and December 21.

The picture for north-facing slopes is somewhat different. At the latitude of the southern Appalachians, where the sun approaches the zenith on June 21, 20-percent north slopes receive almost as much radiation in the summer as 20-percent south slopes. On 100-percent north slopes, however, the radiation at noon on that date is 0.38 of the maximum, as compared with 0.58 on south slopes of the same steepness. On December 21, 100-percent north slopes are in complete topographic shade, whereas 100-percent south slopes receive about 0.48 of maximum radiation at noon.

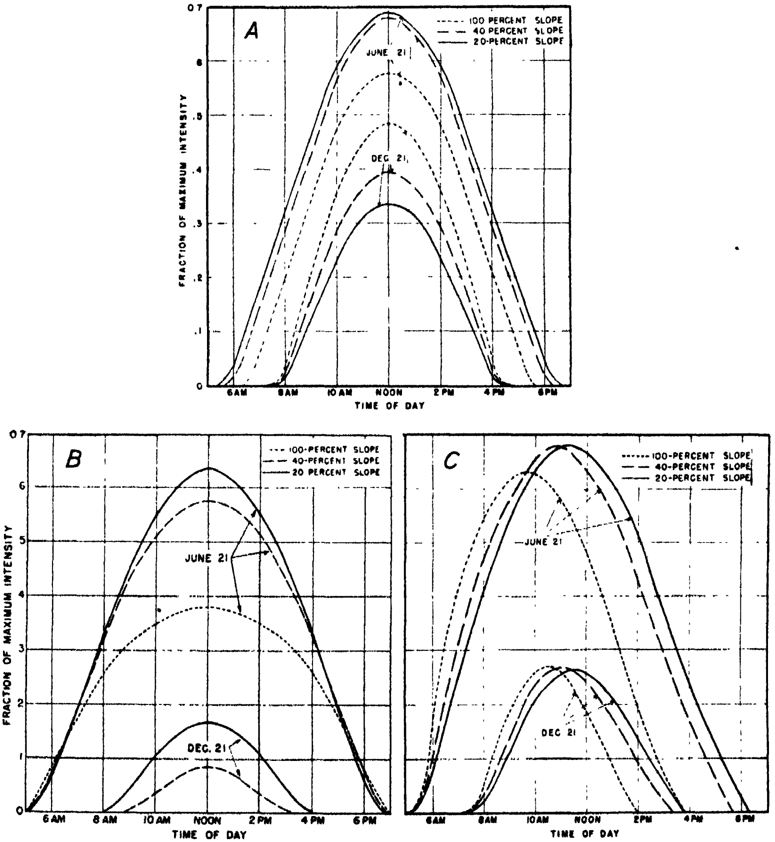

FIGURE 5.—Intensity of radiation received at different times of day on (*A*) south, (*B*) north, and (*C*) east slopes in the southern Appalachians, on June 21 and on December 21.

On east slopes, radiation intensity reaches its maximum before noon. For west slopes the curves would be mirror images of those for east slopes, the maxima occurring in the afternoon.

An example of the calculation of *I*, effective radiation received on a given surface, is presented as a guide to technicians who wish to adapt equations (1) to (5) to other regions of the United States.

Problem: To find radiation I, for a 20-percent east slope at 10 a.m. on June 21 at a north latitude of 35°30′.

Given: Hour angle $h=60°$ (measured from 6 a.m.).
Latitude $\phi=35°30'$ (assumed).
Aspect angle $\beta=90°$ (measured from north).
Angle of slope $\alpha=11°19'$ (for an assumed 20-percent slope).
Solar declination $\delta=23°30'$ (from solar ephemeris tables).
Solar constant $I_0=1$.
Absorption factor $p=0.7$ (assumed).

The following computations are sufficient for determining I. The sines and cosines of A and Z are found from equations (1) and (2) respectively. Sin θ is computed by means of equation 4 from the sines and cosines of A, Z, α, and β. The radiation I is then computed from equation (5). The details of these steps are as follows:

$$\text{Sin } A=\cos 23°30' \sin 60° \cos 35°30'+\sin 35°30' \sin 23°30'$$
$$=.878, \text{ and } \cos A=.478.$$

$$\text{Cos } Z=\frac{\cos 23°30' \cos 60°}{.478}$$
$$=.958, \text{ and } \sin Z=.287.$$

$$\text{Sin } \theta=.878 \cos 11°19'-.478 \sin 11°19' \, (.287 \cos 90°-.958 \sin 90°)$$
$$=.951.$$

$$I=.951\ (.7)^{\frac{1}{.878}}$$
$$=0.63 \text{ of maximum possible radiation.}$$

The value 0.63 thus derived checks with that plotted in figure 5, for the conditions assumed. To obtain the value I in calories per square centimeter per minute, 0.63 would be multiplied by the solar constant given by Forsythe, 1.94.

SOLAR RADIATION AND EQUILIBRIUM FUEL MOISTURE

To determine the quantitative relation between solar radiation and fuel moisture it was necessary to analyze the relations between temperatures and humidities of the fuel bed and the independent variables, air temperature, air humidity, wind, and insolation.

RELATIONS OF RADIATION AND WIND TO TEMPERATURE DIFFERENCE OF FUEL AND AIR

If $\frac{dQ_l}{dt}$ is the rate at which a fuel sample in sunlight loses heat and $\frac{dQ_g}{dt}$ is the rate at which it gains heat, then the temperature of the fuel sample remains stationary only if

$$\frac{dQ_l}{dt}=\frac{dQ_g}{dt} \qquad (6)$$

According to Newton's law of cooling, the rate of loss of heat from an object is directly proportional to the temperature difference existing between the object and its surroundings, and includes rates of loss due to free convection, conduction, and radiation. If the temperature difference is not very great, the radiation loss is small compared with the loss due to convection and conduction. This law can be generalized to give the rate of loss due to forced convection when the object is ventilated by an appreciable wind. If it is assumed that rate of loss of heat from forest fuels is directly proportional to wind velocity, it is possible to write the equation

$$\frac{dQ_l}{dt}=(aV+b)(T_f-T_a) \tag{7}$$

where V is wind velocity, T_f is fuel temperature, T_a is air temperature, and a and b are constants. The values of the constants a and b depend to a considerable extent on the nature of the fuel bed and the manner in which T_f is measured. In this study, for example, the value for T_f, the temperature of the air in the spaces under the topmost individual leaves, is considerably less than would be shown by a thermocouple with the junction cemented to the upper surface of a leaf. In addition, considerable heat is lost by conduction through the fuel bed into the ground. Radiation losses from forest fuels are small.

If I is the intensity of radiation in calories per square centimeter per minute as measured in the artificial-sun apparatus, and if this is the only source of heat, then from equations (6) and (7)

$$\frac{dQ_l}{dt}=\frac{dQ_g}{dt}=I=(aV+b)(T_f-T_a) \tag{8}$$

from which

$$T_f-T_a=\frac{I}{aV+b} \tag{9}$$

The relation between T_f-T_a and I for different wind velocities as established experimentally with the artificial-sun apparatus, is shown in figure 6 in which measured values of T_f-T_a are plotted against

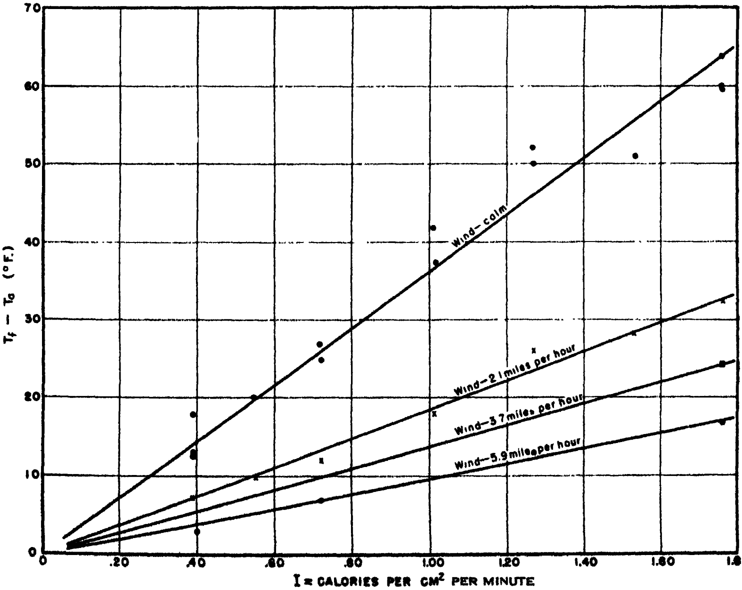

FIGURE 6.—Relation of radiation intensity I and wind velocity V to difference between fuel temperature and air temperature T_f-T_a, as established experimentally with the artificial-sun apparatus.

TABLE 1.—*Experimental data used in determining relation of T_f-T_a to radiation intensity and wind velocity*

Radiation intensity, I (calories per cm² per minute)	Wind velocity, V	Air temperature, T_a	Surface fuel temperature, T_f	Temperature difference, T_f-T_a	Radiation intensity, I (calories per cm² per minute)	Wind velocity, V	Air temperature, T_a	Surface fuel temperature, T_f	Temperature difference, T_f-T_a
	M. p. h.	°*F.*	°*F.*	°*F.*		*M. p. h.*	°*F.*	°*F.*	°*F.*
0.39	0	79	92	13	1.01	2.1	84	102	18
.39	0	78	91	13	1.01	3.7	77	94	17
.39	0	79	97	18	1.27	0	74	124	50
.39	2.1	79	86	7	1.27	0	70	122	52
.39	5.9	79	82	3	1.27	2.1	74	100	26
.56	0	90	110	20	1.27	5.9	70	83	13
.56	2.1	90	100	10	1.53	0	75	126	51
.72	0	80	107	27	1.53	2.1	75	103	28
.72	0	84	109	25	1.76	0	77	137	60
.72	2.1	84	96	12	1.76	0	80	140	60
.72	5.9	78	85	7	1.76	0	83	146	63
1.01	0	77	119	42	1.76	2.1	76	108	32
1.01	0	83	120	37	1.76	5.9	83	100	17

measured values of I. The ranges in air temperature, fuel temperature, wind, and radiation under which observations were made are as follows: Air temperature, 74° to 90° F.; fuel temperature, 83° to 146° F.; wind, 0 to 5.9 miles per hour; radiation intensity, 0.39 to 1.76 calories per square centimeter per minute. Data forming the basis for figure 6 are given in table 1. Computing the constants a and b by the method of least squares gives for equation (9), when T_f-T_a is in degrees Fahrenheit,

$$T_f-T_a=\frac{I}{0.015\ V+0.026} \tag{10}$$

which adequately fits the experimental data. When a and b are determined directly from the data, it is not necessary to know the total emissivity or absorption factor of the fuel bed. It should be emphasized that, in fuel types in which loss of heat to the soil underlying the litter and loss to the air proceed at faster or slower rates than in the beds of hardwood leaf litter used in this investigation, other values for the constants a and b would be obtained.

RELATIONS OF RADIATION, HUMIDITY, AND WIND TO FUEL MOISTURE

The relation of radiation and relative humidity to fuel moisture can most easily be determined by means of the vapor pressure function. If the range of temperature is not too great, the saturated vapor pressure P_s in a given space at a temperature T is related to T approximately by the empirical equation

$$P_s=Ke^{cT} \tag{11}$$

where P_s is in millimeters of mercury, T is in degrees Fahrenheit, e is the base of natural logarithms, and K and c are constants. Table 2 shows the saturated vapor pressure of water for temperatures from 32° to 122° F. and corresponding values of Ke^{cT}, when K and c have values of 1.77 and 0.033, respectfully. That these assumed values provide a close approximation of P_s is seen by comparing the second and third columns in the table; the values agree closely except at high temperatures. The values of K and c in equation (11) could be altered slightly throughout the temperature range of, say, 60°

to 150°, which would be more suitable for use in summer, when fuel and air temperatures are high. This adjustment is unnecessary, however, because through a temperature range of 100° equilibrium fuel moisture values are changed only to the extent of about one-third of 1 percent by errors in the constant c. The constant K does not enter into determinations of fuel moisture equilibria.

TABLE 2.—*Saturated vapor pressure of water for temperatures 32° to 122° F. and corresponding values of Ke^{cT} when $K=1.77$ and $c=0.033$*

Temperature (°F.)	Saturated vapor pressure of water	Ke^{cT}	Temperature (°F.)	Saturated vapor pressure of water	Ke^{cT}
	Mm. of Hg	*Mm. of Hg*		*Mm. of Hg*	*Mm. of Hg*
32	4.58	5.09	86	31.82	30.22
41	6.54	6.85	95	42.18	40.69
50	9.21	9.22	104	55.32	54.76
59	12.78	12.40	113	71.88	73.70
68	17.54	16.69	122	92.51	99.18
77	23.76	22.47			

The vapor pressure P existing when the space is not saturated is related to P_s by the equation

$$P=HP_s \tag{12}$$

where H is the relative humidity. Hence, if P_a is the pressure of the water vapor in a given space at air temperature T_a and relative humidity H_a,

$$P_a=H_aKe^{0.033T_a} \tag{13}$$

and if P_f, T_f, and H_f refer to the same properties in a space immediately adjacent to the fuel particles,

$$P_f=H_fKe^{0.033T_f} \tag{14}$$

But when fuel moisture is in equilibrium, P_a and P_f are equal, hence (13) and (14) can be combined, which gives

$$\frac{H_f}{H_a}=e^{-0.033\ (T_f-T_a)} \tag{15}$$

Substituting (10) in (15) gives

$$\frac{H_f}{H_a}=e^{-I/(0.39V+0.85)} \tag{16}$$

Solutions for $\frac{H_f}{H_a}$ can be found from the nomographic chart figure 7. If any given values of V and I are connected with a straight edge, solutions for the ratio $\frac{H_f}{H_a}$ are given by the intersection of the straight edge with the line representing $\frac{H_f}{H_a}$. The value of H_f can be found from the ratio if H_a is known.

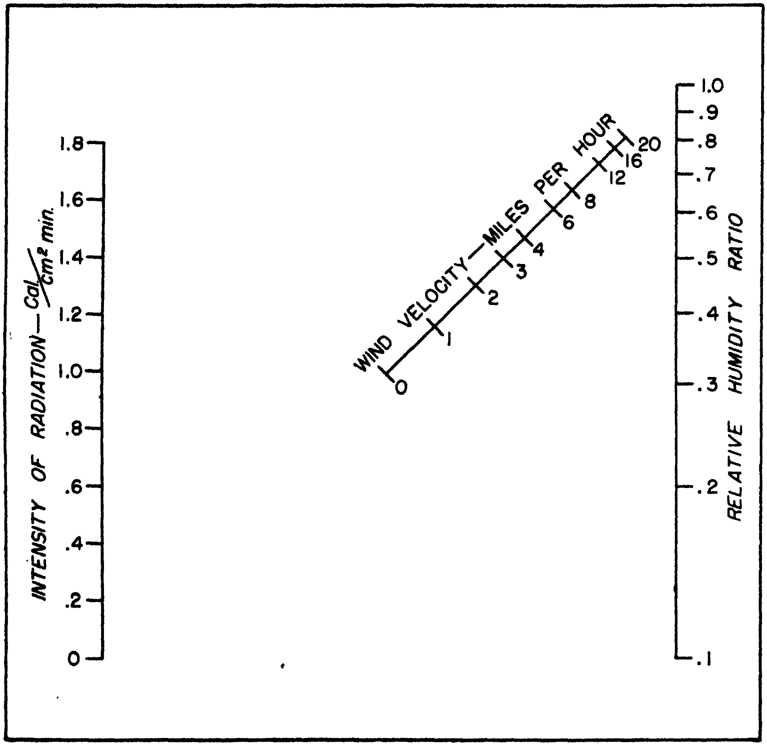

FIGURE 7.—Nomographic chart for solving the equation

$$\frac{H_f}{H_a}=e^{-I/(0.39V+0.85)}$$

The equilibrium moisture content of forest fuels (and cellulose materials in general) is determined chiefly by relative humidity, although temperature has considerable influence. From figure 8, which applies to any finely divided fuel,[6] equilibrium fuel moisture content can be found if fuel temperature T_f and fuel humidity H_f are known. With no sunlight, or if the fuel is completely shaded, fuel temperature and humidity are approximately the same as air temperature and humidity. Under either of those conditions, figure 8 may be used to find equilibrium fuel moisture by considering that $T_f=T_a$ and that $H_f=H_a$.

It is possible to determine fuel moisture equilibria for any given combination of air temperature, relative humidity, wind, and radiation by computing the effective temperature T_f and effective humidity H_f and applying these to figure 8. For example, what is the fuel moisture equilibrium when air temperature is 80°F., atmospheric relative humidity is 30 percent, wind velocity is 0, and radiation intensity is

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[6] Except for high moisture contents, figure 8 checks closely with a fuel-moisture equilibrium chart for Sitka spruce wood given by L. F. Hawley (*10, p. 8*), and with a series of similar relations for several kinds of light fuel reported in the following: DUNLAP, M. E. THE RELATION OF HUMIDITY TO THE MOISTURE CONTENT OF FOREST FIRE FUELS. U. S. Forest Serv., Forest Prod. Lab. 9 pp., illus. 1924. (Unpublished).

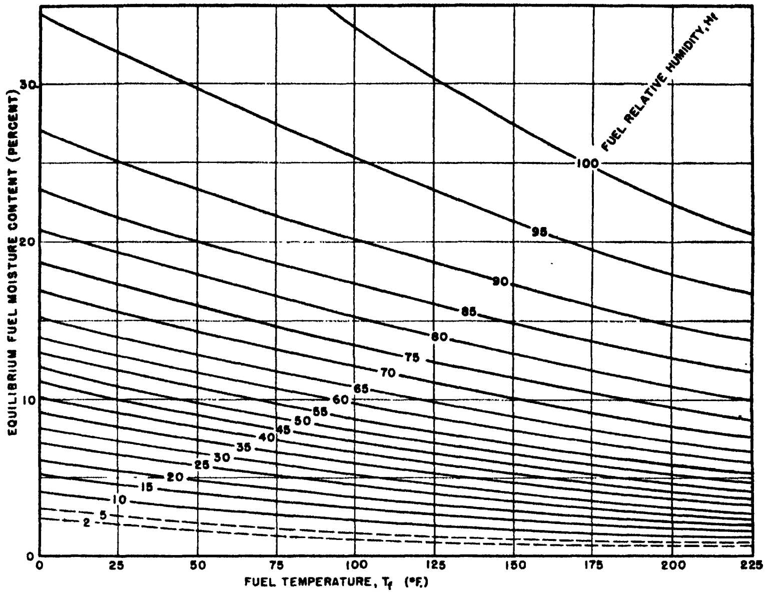

FIGURE 8.—Chart for determining equilibrium fuel moisture content on the basis of fuel humidity and fuel temperature. (Adapted from chart based on results of experimental work by the U. S. Forest Products Laboratory and published in the following: GRAY, L. G. PRELIMINARY REPORT ON FIRE HAZARD RATING STUDY. U. S. Weather Bur. San Francisco. 1933. [Processed.] The broken lines have been added by extrapolation.)

0.80 calories per square centimeter per minute on a slope fully exposed to the sun? From figure 6 the difference between litter and air temperature (T_f–T_a) is found to be 29°, which means that effective or fuel temperature is 109°. The relative-humidity ratio $\frac{H_f}{H_a}$ is found from figure 7 to be approximately 0.4. Effective humidity (at fuel surface) is then easily found; since

$$\frac{H_f}{H_a}=0.4 \text{ and } H_a=30$$

$$H_f=12 \text{ percent}$$

When 109° and 12 percent are used in figure 8 as the values for T_f and H_f, respectively, equilibrium fuel moisture is seen to be about 2.5 percent.

Under the conditions assumed above—air temperature of 80° F. and humidity of 30 percent—but with radiation intensity of 0, equilibrium fuel moisture as determined from figure 8 would be 6 percent. Thus, under these air conditions, where fuel in the sunlight would have an equilibrium moisture content of 2.5 percent, fuel in shade would have one of 6 percent.

Close agreement between equilibrium fuel moistures experimentally determined and those computed from figures 7 and 8 is indicated by the data presented in table 3. The experimental values are from 0

TABLE 3.—*Comparison of experimentally and theoretically determined fuel moisture equilibria for different combinations of radiation, wind, temperature, and humidity*

THIN BASWOOD SLATS

Radiation intensity, *I* (calories per cm.² per minute	Wind velocity, V	Surface fuel temperature, T_f	Air humidity, H_a	Humidity ratio, H_f/H_a	Fuel humidity, H_f	Equilibrium fuel moisture	
						Theoretical [1]	Experimental [2]
	M.p.h.	*°F.*	*Percent*		*Percent*	*Percent*	*Percent*
0.39	0	97	78	0.62	48	7.7	7.7
.39	2.1	86	78	.80	62	11.0	11.1
.56	0	110	54	.52	28	4.3	5.3
.56	2.1	100	54	.71	38	6.2	6.6
.72	0	109	30	.42	13	2.5	3.6
.72	2.1	96	30	.64	19	3.6	4.7
1.01	0	120	50	.30	15	2.6	3.8
1.01	2.1	102	50	.55	28	4.7	5.4
1.27	0	124	60	.22	13	2.5	3.6
1.27	2.1	100	60	.48	29	5.2	5.3
1.53	0	126	58	.17	10	2.0	2.9
1.53	2.1	103	58	.40	23	4.2	5.3
1.76	0	137	56	.13	7	1.8	3.6
1.76	2.1	108	56	.34	19	3.5	4.3
1.76	0	135	60	.13	8	1.9	3.0
1.76	3.7	102	60	.58	35	5.9	5.9
1.76	0	146	54	.13	7	1.5	2.4
1.76	5.9	100	54	.57	31	5.2	6.4

HARDWOOD LEAF LITTER

Radiation intensity, *I* (calories per cm.² per minute	Wind velocity, V	Surface fuel temperature, T_f	Air humidity, H_a	Humidity ratio, H_f/H_a	Fuel humidity, H_f	Theoretical [1]	Experimental [2]
1.76	0	160	47	0.13	6	1.8	2.8
1.76	5.7	98	47	.55	26	4.6	5.6
1.76	0	125	53	.13	7	2.0	2.7
1.76	6.5	91	53	.58	31	6.0	7.0

[1] Computed from figures 7 and 8.
[2] Determined by use of the artificial-sun apparatus.

to 1.8 percent higher than those computed from the graphs; the average departure is 0.8 percent. Undoubtedly, this difference was due partly to a slight lag in rate of drying but mostly to hysteresis.

EFFECT OF WIND ON MOISTURE EQUILIBRIA OF IRRADIATED FUELS

From equation (16) one would be inclined to deduce that fuels exposed to both radiation and wind have higher equilibrium moisture contents than fuels exposed to radiation in still air. Although contrary to the usual ideas of drying phenomena, this deduction is conclusively borne out by the fuel moisture curves in figures 9, 10, and 11, and by the data presented in table 3. Figure 9, based on data for thin basswood slats immersed in water and then exposed in artificial-sun apparatus, shows that the slats had higher equilibrium moisture contents in wind than in calm air. Figure 10 indicates a similar contrast between slats in wind and in calm air that were started at oven dryness. Figure 11 shows the rate of drying of hardwood leaf litter having an initial moisture content of about 20 percent, a value within the inflammable range. This fuel responds in a manner identical to that of the wood slats represented in figures 9 and 10.

The higher moisture equilibria of irradiated surface fuels subjected to wind are due to the higher humidities associated with lower fuel temperatures. For irradiated fuels the cooling action of wind more than offsets its drying action. Figure 10 demonstrates that a cool fuel can absorb and hold more moisture than a hot one. (This is a partial explanation for the fact that higher moistures exist on cut-over areas bearing residual stands than on comparable clear-cut areas.)

The general conclusion may be drawn that, in regions where forest fuels are normally exposed to sunlight, higher fuel moisture equilibria are associated with clear windy weather than with clear calm weather. The difference may be as great as 6 percent, depending chiefly on levels of the atmospheric factors temperature, humidity, and wind. It is greatest for the range of fuel moisture below about 15 percent. This influence of wind operates only to a slight extent on steep north slopes, where insolation is low.

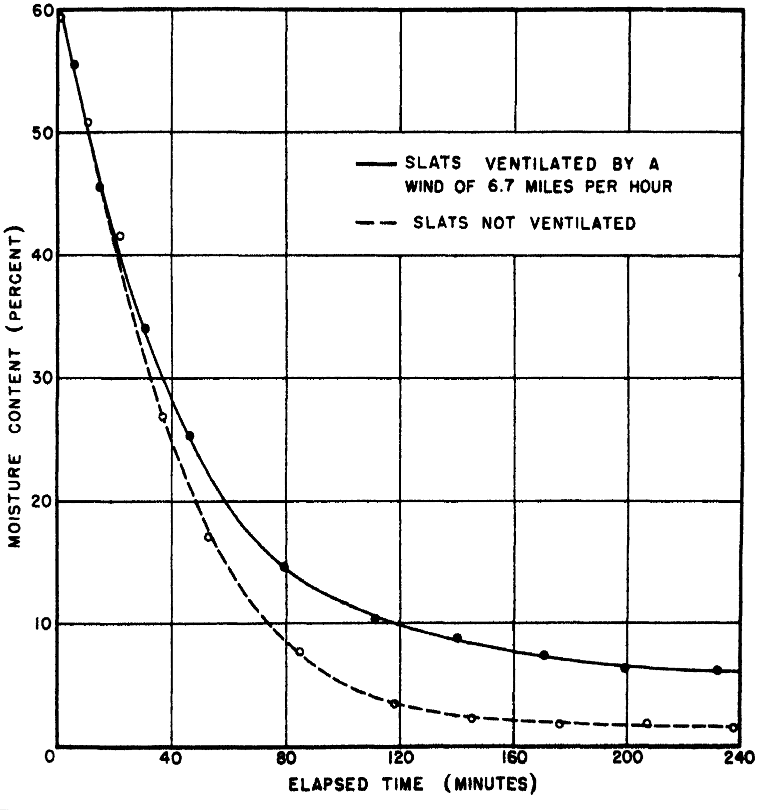

FIGURE 9.—Drying curves for irradiated wood slats exposed in calm air and for others exposed to a wind of 6.7 miles per hour. Slats were initially immersed in water. Relative humidity during test, 55 percent; air temperature, 80° F.; radiation, 1.76 calories per square centimeter per minute.

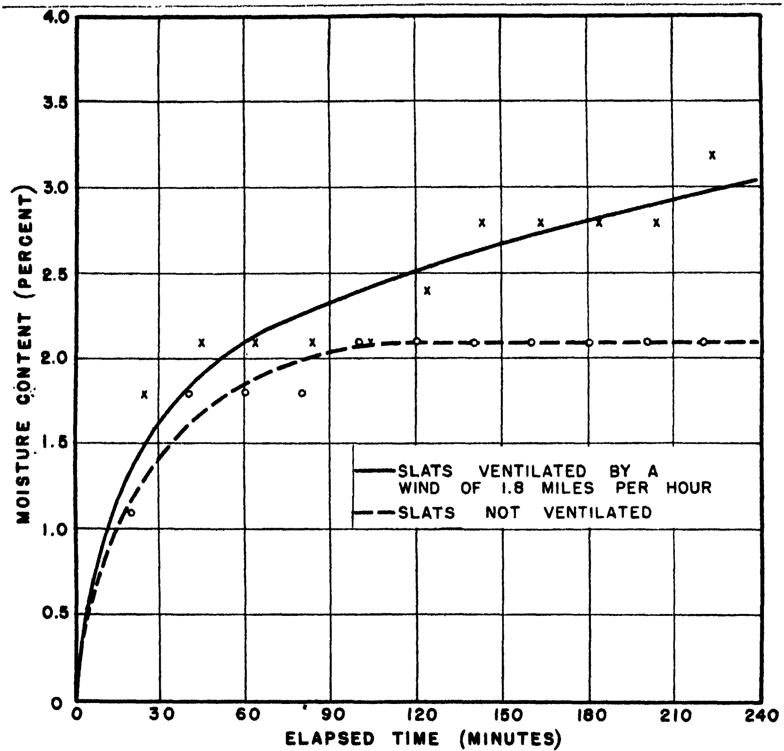

FIGURE 10.—Curves showing effect of wind on rate of gain of moisture by wood slats initially oven-dry and exposed to a radiation of 1.76 calories per square centimeter per minute. Relative humidity during test, 62 percent; air temperature, 82° F

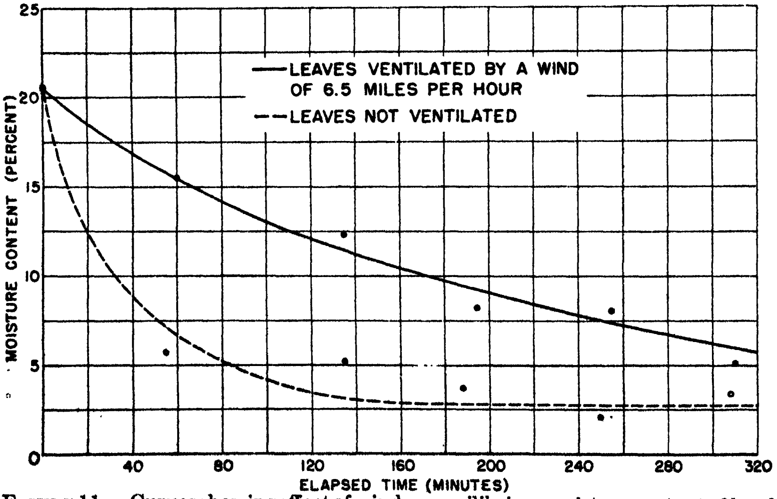

FIGURE 11.—Curves showing effect of wind on equilibrium moisture content of hardwood leaf litter exposed to a radiation of 1.76 calories per square centimeter per minute. Relative humidity during test, 51 percent; air temperature, 84° F.

SOLAR RADIATION AND RATE OF DRYING OF FOREST FUELS

As has been stated, the moisture content of the surface forest litter in the southern Appalachian region may be considered as being in the equilibrium state most of the time. The moisture content of lower layers of forest fuels, on the contrary, is not often in equilibrium with the humidity of the air immediately above the fuel bed; and it has almost as great an influence as that of the surface fuels in determining the inflammability of the total fuel layer. The rate at which subsurface fuels dry out after a rain is therefore particularly important. Only after determining the principles that govern this rate of loss of moisture can fire-research specialists supply fire dispatchers with guides that will enable them to judge when fuel is approaching an inflammable state. A complete solution of the rate-of-drying problem would find more immediate application than one of any other phase of fuel moisture studies.

Complete solution of the rate-of-drying problem is outside the scope of the present investigation, but this problem deserves discussion in connection with a general analysis of the factors upon which fuel moisture content depends. For the benefit of other investigators, some of the theoretical concepts of rate of drying of forest fuels will be outlined.

If q is the moisture content of a thin layer within a thick (about 2-inch) bed of fuel, the rate of loss of moisture from this layer should be given with reasonable accuracy by a differential equation of the form

$$\frac{dq}{dt}=-f_1(q)(P_f-H_aP_s)f_2(V)f_3(D) \tag{17}$$

where P_f is the pressure of water vapor within the fuel layer, P_s the pressure of saturated water vapor at air temperature, H_a the relative humidity of the air at the surface of the fuel bed, $f_1(q)$ some undetermined function of fuel moisture, $f_2(V)$ some function of wind velocity V, and $f_3(D)$ some function of the thickness D of the fuel above the selected thin layer.

The evaporating force is proportional to the term $(P_f-H_aP_s)$, but the rate of evaporation depends also on the vapor pressure gradient existing between the fuel layer and the space immediately above the surface of the fuel bed. The vapor pressure gradient is determined in part by the thickness and porosity of the fuel above the layer under consideration, and in part by wind velocity. The rate of evaporation depends in some complex way on the water supply or on $f_1(q)$. The vapor pressure P_f in the fuel layer is a function of the temperature of the fuel layer, which in turn is determined by air temperature, wind velocity, intensity of radiation, and rate of evaporation $\frac{dq}{dt}$. If a temperature gradient exists in the fuel bed, P_f depends also on D. If the moisture content q is below the fibre saturation point (about 30 percent) P_f is a function of q, since the water molecules in that case become partially bonded to the molecules of wood substance and this causes a decrease in P_f. For this reason equation (17) cannot easily be integrated if q is less than 30 percent. For values of q above the fibre saturation point, equation 17 can be integrated as follows, provided that the rate of loss of heat due to evaporation cooling is small compared with the intensity of radiation:

$$\int_{q_o}^{q} \frac{dq}{f_1(q)} = -(P_f - H_a P_s) f_2(V) f_3(D) \int_{o}^{t} dt$$

or

$$F(q_o) - F(q) = (P_f - H_a P_s) f_2(V) f_3(D) t \tag{18}$$

where $F(q)$ is the integral of $f_1(q)$

To find the time required for two similar layers of fuel to lose equal amounts of water under different intensities of radiation and wind velocities, it is possible to write

$$F(q_0) - F(q) = (P_f - H_a P_s) f_2(V) f_3(D) t = (P'_f - H_a P_s) f_2(V') f_3(D) t'$$

or

$$(P_f - H_a P_s) f_2(V) t = (P'_f - H_a P_s) f_2(V') t' \tag{19}$$

where P'_f, V', and t' are fuel vapor pressure, wind velocity, and time, respectively, for the second fuel layer. P_f and P^1_f must be the saturated vapor pressures corresponding to the fuel temperatures, and should be regarded as the vapor pressures existing on the surface of individual wet leaves in the fuel layers. After substitution of the exponential relation (equation 11) for vapor pressures and simplification, equation (19) becomes

$$[e^{c(T_f - T_a)} - H_a] f_2(V) t = [e^{c(T'_f - T_a)} - H_a] f_2(V') t'$$

Substituting $\frac{I}{aV+b}$ for $T_f - T_a$ (equation 9) gives

$$\left[e^{\frac{cI}{aV+b}} - H_a\right] f_2(V) t = \left[e^{\frac{cI'}{aV+b}} - H_a\right] f_2(V') t' \tag{20}$$

When an assumed wind velocity value of 2.5 miles per hour was inserted in equation (19) along with the values of a, b, and c previously given, the equation was simplified to

$$(e^{0.54I} - H_a) t = (e^{0.54I'} - H_a) t' \tag{21}$$

Values computed from this equation for relative time required for thin layers of fuel near the surface of fuel beds on north and south slopes to lose equal amounts of moisture after a rain are presented in table 4.[9]

TABLE 4.—*Relative drying time*[1] *of fuel near surface of fuel bed on north and south slopes in May and November*

Slope (percent)	May 6		November 6	
	North	South	North	South
20	2.5	2	7	4
40	2.5	2	9	3.5
60	3	2	15	3.5

[1] Calculated by use of equation (21). Wind velocity of 2.5 m. p. h. and 40 percent relative humidity are assumed. Values may be regarded as either days or hours.

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[9] According to the current results of a study to determine the effect of terrestrial radiation on fuel moisture, on most forest areas the ratios of time required for drying on north slopes to that required on south slopes may be considerably greater than indicated in table 4. Under some conditions, north slopes may actually gain moisture on calm, cloudless days and nights while adjacent south slopes continue to lose moisture. Equation (17) is sufficiently general to include in its solution the effects of terrestrial radiation on fuel moisture.

The tabular value of *t*, relative drying time, for a given slope and season of year is the reciprocal of the area between two curves plotted on the same graph, one representing hourly values of the quantity $e^{0.541}$, the other representing values of relative humidity H_a drawn as a smooth curve from 100 percent at sunrise to 40 percent at noon to 70 percent at sunset. It was assumed that no significant drying occurred during the night. The area between the two curves represents the integrated value of $e^{0.541} - H_a$ for the daylight hours.

Experimental data obtained with the artificial-sun apparatus show

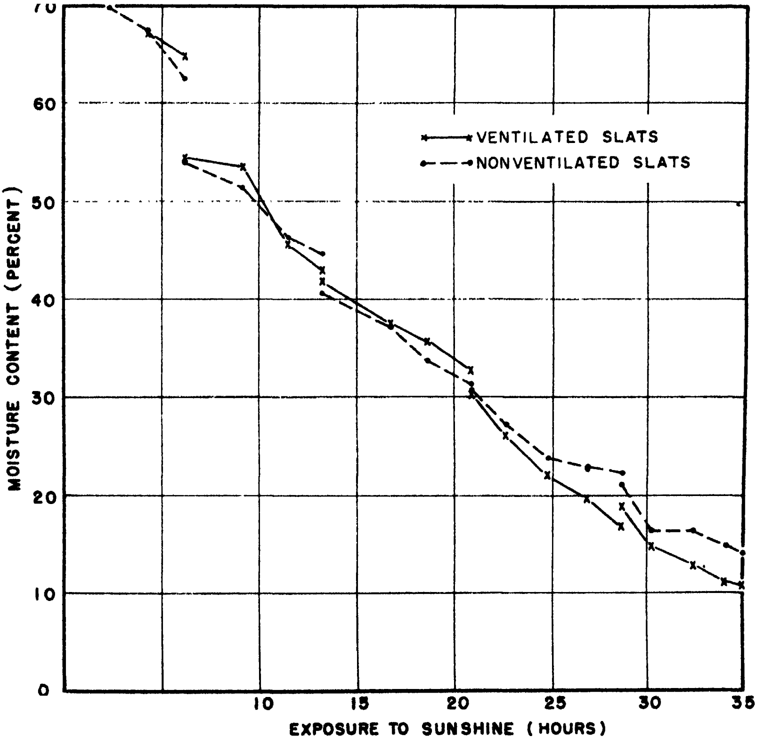

Figure 12.—Drying curves for lower layers of fuel exposed to radiation, as measured by thin slats buried in the lower layers of litter. (Breaks in curves indicate effect of turning off fans and lights in artificial-sun apparatus at night.)

that, in bright sunlight, winds of low velocities (1 to 3 miles per hour at the fuel surface) have no appreciable net effect on rate of drying of subsurface fuel. This is illustrated by drying curves for the lower fuel presented in figure 12. The same thing is true of basswood slats of high moisture content, as is shown by the portion of figure 9 representing moistures above the fibre saturation point. In this figure it will be noted that when their moisture content was above 30 percent, the slats dried at almost the same rate in calm air as in a wind of 6.7 miles per hour. A logical explanation is that although wind has a pronounced tendency to hasten drying this is offset, when wind velocity is low and radiation intensity is high, by its tendency to cancel the influence of radiation on temperature, just as is the wind's tendency to lower the equilibrium moisture content of surface fuels. Figure 13, which shows the effect of wind on the rate of drying of basswood slats in the absence of radiation, indicates that this explanation is correct, because the fuel in wind dried faster than that in calm air.

Probably several other factors influence the form of the basic equation (18), and the values presented in table 4 may be only rough approximations. For example, the moisture gradient within a drying fuel bed is affected by the loss of moisture from all fuel layers, not just one layer. However, equations (19) to (21) probably would not be changed appreciably by variation in the moisture gradient. A vertical temperature gradient also exists within a fuel bed, and T_f-T_a is smaller for lower fuel layers than for layers near the surface. For thin basswood slats exposed so that the air can circulate freely about them, the rate of heat loss due to evaporation cooling is not small compared with the intensity of radiation received; it may occasionally be almost half as great as the radiation intensity. However, evaporation cooling is probably much less in fuel beds.

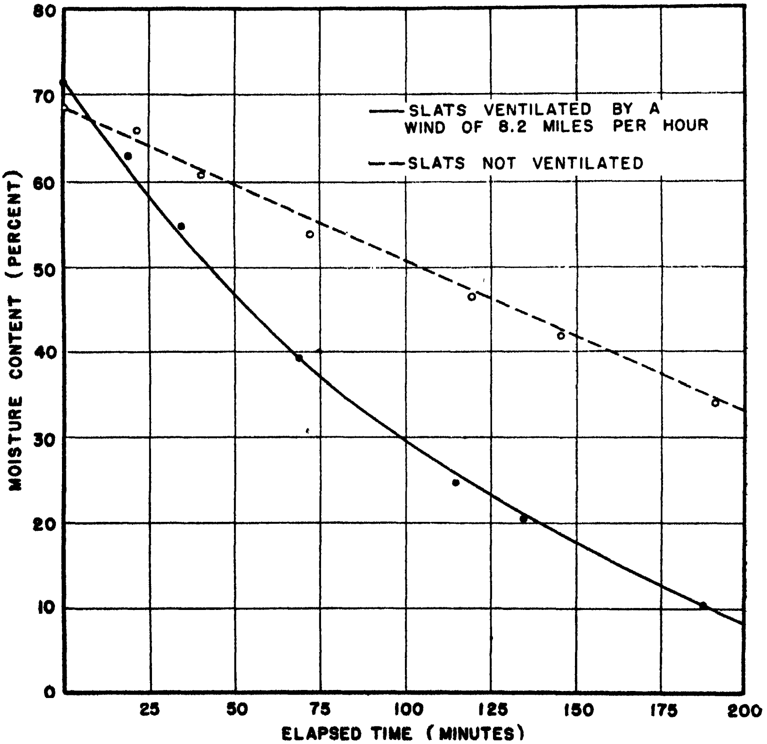

FIGURE 13.—Rates of drying of ventilated and nonventilated wood slats in absence of radiation.

The development of rate-of-drying equations for moisture contents below the fibre saturation point is a phase of the problem that has received relatively little attention from technical foresters, but is one of extreme importance.

Work on the entire rate-of-drying problem is continuing at the Appalachian Forest Experiment Station.

SIGNIFICANCE AND APPLICATION OF FINDINGS

As was mentioned in the introduction, fire-danger ratings are used as guides in the employment and disposition of fire-control personnel. When the weather becomes dry and windy, more lookout men and suppression crews are required—especially in the drier and windier parts of a forest. Danger ratings help fire-control administrators to estimate how many men they need and to distribute their forces advantageously. In order to increase the refinement with which forest fire-danger ratings are made and applied, one necessity is to gain a better understanding both of the influence of topography and of "season of the year" on fire danger. In the United States the significance of seasonal changes in radiation intensity is particularly great in northern latitudes; in hardwood forest types that are leafless during late spring, when radiation is intense; on areas of rugged topography; and on western burned-over areas where dead forest fuels are exposed to sunlight during the summer months. This paper provides technicians in all regions with a general technique and equations for computing moisture equilibria of surface fuels for any combination of date, hour, slope, aspect, air temperature, air humidity, and wind, and with information on some of the basic relations governing rate of drying. This should lead to a better understanding regarding these factors on the part of workers engaged in perfecting fire-danger rating systems. Eventually, by acquiring and using a knowledge of radiation effects, it should be possible to modify the several measurements made at a skeleton network of fire-danger stations to fit all topography adjacent to the stations, thus greatly reducing the labor and cost of danger measurements.

Rates of drying of forest fuels calculated in this study can be used as a partial correction for ratings of fire danger at key stations, to make them apply to areas where conditions cannot be measured. Table 4, compiled in connection with the theoretical development of rate-of-drying relations, brings out differences in rate of drying for several aspect-slope-season combinations. After a rain in early May, it indicates, the fuel on a 60-percent south slope in the southern Appalachians loses as much moisture in 2 days as that on a corresponding 60-percent north slope loses in 3 days. Likewise in November the fuel on the south slope dries from a saturated condition down to an inflammable one in 3½ days, but the fuel on the north slope does not reach this point until after 15 days of the same type of weather.

Rate-of-drying data will become especially valuable to fire-control administrators when more is learned about variations in effect of wind corresponding to topographic variations and about variations in temperature and humidity corresponding to variations in elevations. Hayes (*11*) has worked on the latter problem in the northern Rocky Mountains, and similar studies have been in progress for 3 years in the southern Appalachians. Thus far, the most important variation isolated in these studies is that of ground wind velocity with elevation and aspect.

An example will indicate to fire specialists how the findings of this study may eventually be applied, at the same time indicating deficiencies in present knowledge and a direction for future work.

A small portion of the Pisgah Ranger District of the Pisgah National Forest, in western North Carolina, has been used for this illustration. For May 6 and November 6, near the peak of the spring and fall fire seasons, a typical set of conditions has been assumed; namely, air temperature of 70° F., relative humidity of 40 percent, no wind at the litter surface, and the hour of 2 p. m., when the moisture content of light fuels is likely to be close to the equilibrium point. Usually, on those dates, a typical hardwood forest at the average elevation of the Pisgah district, about 4,000 feet, is leafless and a maximum of sunlight penetrates the stand and reaches the fuels of the forest floor.

To illustrate in detail the variations in fuel moisture equilibria associated with topography, due to unequal insolation, lines passing through points of equal surface fuel moisture equilibria as determined according to the findings of this study have been superimposed on an enlarged contour map of this area, which is roughly 2½ miles square, for May 6 and November 6 (fig. 14). The variation brought out emphasizes the problem confronting one who must locate fire-danger stations to sample "representative" conditions of fuel moisture. Knowledge of this variation, however, should help him choose locations that meet the specifications set up. The spread in equilibrium fuel moisture on November 6 is 5 percent, a difference particularly significant in such a low range of fuel moisture as that represented here. On May 6 fuel moistures are lower and there is less difference between north- and south-facing slopes, because the sun is higher and its rays are more effective. .

Table 5, showing the relative portions of the area on which equilibrium surface fuel moisture content was theoretically of given classes on the two dates, emphasizes the significance of the seasonal differences brought out in figure 14. In the southern Appalachian system of danger rating, fuel moisture of less than 4 percent is classed as critical. In May, equilibrium moisture content falls in this extreme class on 76 percent of the area used in the illustration; in November, under exactly the same atmospheric conditions it does so on only 9 percent. In other words, in November it is likely that fewer fires will start and those that start will spread more slowly and be easier to control on much more of the area than in May, even though all atmospheric conditions are the same for both dates.

Basic information such as that used to construct figure 14 can be tabulated as in table 6 to gain an appreciation of the range in equilibrium fuel moistures that may be expected in mountainous country and to facilitate its use by field men.

TABLE 5.—*Fuel moisture classification of sample area for May 6 and November 6*

Equilibrium fuel moisture content (percent)	Proportion of total area		Equilibrium fuel moisture content (percent)	Proportion of total area	
	May 6	November 6		May 6	November 6
	Percent	*Percent*		*Percent*	*Percent*
Less than 3	53	0	Less than 7		88
Less than 4	76	9	Less than 8		97
Less than 5	96	49	Less than 9		100
Less than 6	100	70			

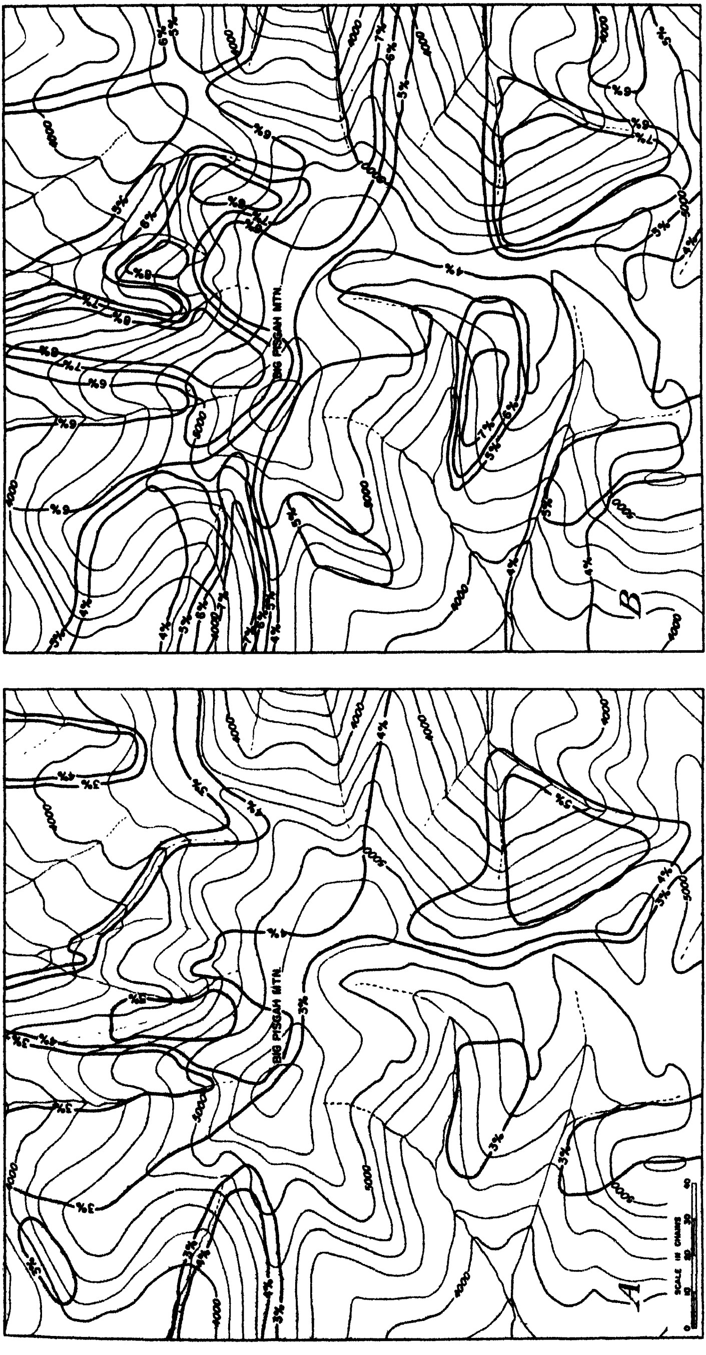

FIGURE 14.—Fuel moisture equilibrium maps for a representative portion of the Pisgah National Forest, N. C.: *A*, May 6; *B*, November 6

TABLE 6.—*Fuel moisture equilibria for sample area, by aspect, slope, and wind class, when air temperature is 70° F., relative humidity is 40 percent, and hour is 2 p. m.*

MAY 6

Aspect[1]	Fuel moisture by steepness of slope (percent) and velocity of wind									
	20		40		60		80		100	
	0 mph	3 mph	0 mph	3 mph	0 mph	3 mph	0 mph	3 mph	0 mph	3 mph
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
N	3	5	3	5	4	6	4	6	4	6
NE	3	5	4	5	5	6	5	6	6	7
E	3	5	3	5	5	6	5	6	5	7
SE	3	5	3	5	4	5	4	5	4	6
S	2	4	2	5	3	5	3	5	3	5
SW	2	4	2	4	2	4	2	4	2	4
W	2	4	2	4	2	4	2	4	2	4
NW	3	5	3	5	3	5	3	5	3	5
NOVEMBER 6										
N	5	7	6	7	7	8	8	8	8	8
NE	5	7	6	7	8	8	8	8	8	8
E	5	6	6	7	6	7	7	8	8	8
SE	4	6	4	6	4	6	4	6	5	6
S	4	6	4	6	3	5	3	5	3	5
SW	4	6	3	5	3	5	3	5	4	5
W	4	6	4	6	4	6	4	6	4	6
NW	5	6	5	6	5	6	6	7	6	7

[1] For flats, the fuel moisture percent is as follows: Wind of 0 mph, 2 percent on May 6, 4 percent on Nov. 6; wind of 3 mph, 5 percent on May 6, 6 percent on Nov. 6.

Obviously, one condition that complicates the application of the findings regarding equilibrium fuel moisture relations to a heterogeneous forest area is the variation in air temperature, air humidity, and wind from one locality to another. Until more is known about such differences, much of the significance of the relation of radiation to equilibrium fuel moisture must lie in the basic theory developed rather than in everyday application by fire-control administrators. Nevertheless, the importance of determining the variations of atmospheric factors with topography becomes more evident when the potential differences in actual rate of spread of fires resulting from the extremes of fuel moisture noted in figure 14 are estimated. The rate-of-spread figures vary not only with atmospheric factors, but also with steepness of slope, fuel type, and perhaps other factors. On the average slope, on a calm day, and in leaf litter typical of the southern Appalachians, fires burning in fuel with 4 percent moisture would increase their perimeter about 4 or 5 chains per hour faster than fire burning in fuel with 8 percent moisture. All other things being equal, a dispatcher recognizing such a difference in fuel moisture would have to send two or three more men to a fire in the drier fuels than to a similar fire in the more moist fuels, if control were to be accomplished in the average length of time.

It must be emphasized that several other considerations influence manpower requirements for fire control to a much greater extent than 4- or 5-percent differences in fuel moisture. It must be remembered also that only for the very light surface fuels is moisture content frequently near equilibrium with that of their surroundings; therefore, the data presented here do not apply to fuels heavier than hardwood leaves, dead grass, and surface layers of pine needles.

SUMMARY

Forest fire-danger rating systems, now in use in all forest regions of the United States, are based chiefly on measurement of wind and of fuel moisture. While many workers have investigated atmospheric and related elements that control fuel moisture, little research has been done on solar radiation and its influence on fuel moisture equilibria and rates of drying. With a view to contributing to the refinement of fire-danger rating systems and application of the ratings, a study of solar radiation and fuel moisture was begun in the southern Appalachians in 1938.

A method has been developed whereby radiation intensity can be determined for any season of year, hour of day, slope, and aspect. Examples are given showing the widely different radiation intensities that are to be expected under different combinations of these factors even though atmospheric conditions are the same. The relation of solar radiation intensity to surface fuel moisture equilibria and, to a lesser extent, its relation to rates of drying have been established on the basis of theory and of data obtained by use of an "artificial sun" apparatus, a specially constructed weather synthesizer. This apparatus permits both field and laboratory observation of moisture equilibria and rates of drying under various combinations of radiation, wind, and humidity. Formulae have been developed so that for any combination of air temperature, relative humidity, and wind velocity, equilibrium moisture content of forest litter can be derived for any season, slope, and aspect. These formulae can be used universally, provided radiation intensities are adjusted for latitude.

The influence of wind on fuel drying is emphasized. In bright sunlight, contrary to popular belief, wind maintains levels of fuel moisture higher than those in calm air. The reason is that for fuels in the sun the wind's cooling action more than offsets its drying action. This is important in some regions where fuels are fully exposed to sunlight during the fire season.

Fuel moisture equilibrium maps are presented showing variations with season, aspect, and slope that result from variations in radiation intensity alone. A table is presented showing differences in drying rates caused by differences in radiation.

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A PRIMARY CAUSE OF DARKENING IN BOILED POTATOES AS REVEALED BY GREENHOUSE CULTURES[1]

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INTRODUCTION

Potatoes that darken after boiling have become increasingly prevalent in Wisconsin in recent years. When blackening in boiled potatoes (*Solanum tuberosum*) first became the subject of study in the fall of 1932 it was thought that some deficiency in soil minerals might be involved, and that this deficiency was probably linked with climatic influences upon both the soil and the plant. Accordingly, a summary of average weather conditions representative of northern (Antigo), central (Hancock), and southern (Madison) Wisconsin for the greater part of the preceding 5-year growing seasons was assembled. Table 1 shows the departures from the normal temperature and precipitation for each of these periods. From these data it is apparent that the growing season for the 3 years 1930–32 was subject to high temperature while the rainfall was generally deficient in all but the southern area. The records for August and September as related to periods of active tuberization are presented in table 2. The records for August show uniformly unfavorable departures from normal in 1930, with Hancock subject to drought in 1931 and Antigo to heat in 1932. The records for September show that this month was excessively dry in Hancock in 1930, excessively hot and wet in all areas in 1931, and droughty in all areas in 1932. The last-named year was the beginning of an era characterized by hot, dry summers which extended through 1939. During this period the discoloration of potatoes after boiling was more evident than before.

TABLE 1.—*Departures from the normal temperature and precipitation for the growing seasons 1928–32, as shown by the monthly averages, June to August, inclusive, at stations representative of northern (Antigo), central (Hancock), and southern (Madison) Wisconsin*

Year	Antigo		Hancock		Madison	
	Temperaature	Precipitation	Temperature	Precipitation	Temperature	Precipitation
	°F.	*Inches*	*°F.*	*Inches*	*°F.*	*Inches*
1928	−1.0	+2.13	−2.2	+1.34	−1.5	+0.9
1929	−.5	−.12	−2.0	+.59	−1.6	+.8
1930	+2.4	−.39	+.7	−.51	+1.3	−.1
1931	+3.2	−1.16	+3.0	−.66	+3.0	+.2
1932	+2.7	−1.71	+2.6	−.59	+2.1	0

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TABLE 2.—*Departures from normal temperature and precipitation for periods of tuberization at stations representative of northern (Antigo), central (Hancock), and southern (Madison) Wisconsin, 1928–32*

Year and month	Antigo		Hancock		Madison	
	Temperature	Precipitation	Temperature	Precipitation	Temperature	Precipitation
	F.	*Inches*	*F.*	*Inches*	*F.*	*Inches*
1928:						
Aug	+1.9	+3.62	+0.6	+2.78	+0.4	+1.5
Sept	−3.8	+2.73	−4.6	+1.07	−3.4	−1.94
1929:						
Aug	+.1	−.83	−2.0	−1.11	+1.3	−2.3
Sept	+1.7	−.96	−1.6	−1.12	−1.8	−.55
1930:						
Aug	+3.5	−2.25	+1.5	−2.39	+2.7	−1.6
Sept	+1.4	−.46	+.4	−2.52	+1.4	+1.07
1931:						
Aug	+.2	−.16	+.4	−.99	+1.0	+2.0
Sept	+6.5	+1.82	+4.9	+2.95	+6.0	+3.45
1932:						
Aug	+3.4	−.75	+1.3	+.32	+1.4	–
Sept	−.7	−1.56	−1.1	−1.66	−.5	−3.54

GENERAL PROCEDURE

The greenhouse experiments described in the present paper were undertaken to supplement observations on the prevalence of tuber discoloration in relation to climatic and fertility variations in the field.

The usual procedure was to break the dormancy of the tubers by heat treatment. This was done usually in December and tuber cuttings were planted in flats of pure quartz sand. Unless specified otherwise, the seed stock was obtained as free from disease as possible by the aid of associates engaged in its certification.[2] As soon as sprouting was general the plants were exposed to natural illumination and supplied with small amounts of the major nutrient elements. At this stage of development, and for several weeks after transplanting, the house was kept cool (below 60° F. at night) to stimulate vigorous vegetative development. Thus the plants available for transplanting were fairly uniform, and the major vegetative activity occurred at a time when increasing intensity of sunlight and length of day were favorable. The maturation of the crop occurred largely in April, while outdoor temperatures were still favorably low. During the early development of the crop the length of day was increased somewhat by operating lamps through an electric time switch, but the intensity of this illumination at the general plant surface did not exceed 200 foot-candles. Shortening of the daylight period was accomplished for the bed cultures by the use of canvas covers. Values of the solar radiation during the several culture periods, based on records of climatic conditions provided by the local United States Weather Bureau office one-half mile from the greenhouse, are given in table 3.

As the work progressed the writers became aware of the need of storing the crop for about 1 month after harvesting before making the blackening test. This interval allows time for the gradual transformations which cause discoloration after cooking. It was also found that halved tubers boiled in the skins should be peeled before grayness was determined in order to allow lateral diffusion of light into the tissue and prevent its absorption. Therefore, all of the boiling records obtained from longitudinal halves of tubers, which antedated the autumn of 1939, have been adjusted in accordance with this finding.

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[2] For this assistance the writers are indebted to Professors J. G. Milward, John W. Brann, and G. H. Rieman, of the Departments of Horticulture, Plant Pathology, and Genetics, respectively.

TABLE 3.—*Values of outdoor solar radiation during the several culture periods in the greenhouse bed and in pots*

Year	Period	Greenhouse bed			Pots		
		Total solar radiation per cm.^{2}	Proportion of normal	Proportion of the June-August normal	Total solar radiation per cm.^{2}	Proportion of normal	Proportion of the June-August normal
		Gram calories	*Percent*	*Percent*	*Gram calories*	*Percent*	*Percent*
1934	Feb. 1-May 14	35,186	104	77			
1935	Mar. 15-June 15	36,092	92	79			
1936	Jan. 15-Apr. 15	26,188	106	57			
	Feb. 1-Apr. 20				24,660	105	54
1937	Jan. 6-Apr. 20	29,349	104	61	29,349	104	61
1938	Jan. 1-Apr. 15				23,639	88	52
	Feb. 1-Apr. 15	19,831	90	43			
1939	Jan. 16-Apr. 20				27,110	103	59

No particular refinement was applied to the boiling procedure, for it was observed that the proportion of water used, its hardness, and the time of boiling had little influence on the final discoloration. The samples were removed before they showed any tendency to disintegrate, and full discoloration occurred in the process of cooling. Ten tubers selected at random in one test were found to give reproducible results, but a later trial has indicated that a larger sample may be necessary for full reliability of the boiling record. The cooked samples were observed against a background of ordinary filter paper under northern skylight and the discoloration is expressed as light gray and medium gray, It was impracticable to compare the grayness of the mashed tissue with photometric standards, as was done by Bilham and associates (*1*),[3] because the preponderant white particles of starch cover the darkly pigmented products.[4] Darkening after boiling is common to regions adjacent to mechanical injury or pathological invasion and therefore bruised or scabby potatoes have been excluded from the samples.

BED PLANTINGS

SEASON OF 1934

A soil medium was prepared in a bed 7 inches deep by mixing about 1 part by weight of Miami silt loam with 4 parts of sand from a local deposit. From data on the composition of these soils it was estimated that the mixture contained not more than 0.05 percent nitrogen, 0.02 percent phosphorus, and 0.50 percent potassium, with 70 pounds of available potassium per acre. This soil lay upon the clay subsoil at the bottom of the bed, so that exchanges of water and nutrients between the two were possible. No fertilizer was applied but a differential of water supply in parts of the bed was established during the last month of culture.

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[3] Italic numbers in parentheses refer to Literature Cited, p. 193.

[4] This explanation was suggested by Edward E. Miller, formerly research assistant, Department of Physics, University of Wisconsin.

In the 1934 test two discoloring seed stocks of Rural New Yorker were compared with a normal one. One of the abnormal stocks had turned quite dark after cooking and the other was classified as "weak." The latter had internal browning and, on cooking turned a bluish black. Sprouts emerged from the normal stock much later than from the abnormal ones and the stems of the latter were somewhat spindling. No artificial illumination was applied to this crop. The yields were substantial from the abnormal stocks but low from the normal one, with general benefit from irrigation. As the boiling test was made soon after the crop was dug, the significance of the result is questionable. All the samples rated acceptably white as of that time.

SEASON OF 1935

In the 1935 test emphasis was placed upon deficiency of potassium in association with drought. The residual soil of the previous season was treated with a general fertilizer about the equivalent to 1,200 pounds to the acre of 3–10–20, in the complete form, which provided a liberal supply of potassium. On one-half the area the added potassium was decreased 90 percent by replacing potassium sulfate with its equivalent of sodium sulfate. Nitrogen was supplied in ammonium sulfate and phosphorus in dicalcium phosphate. The general plan of treatment was as follows:

Low K supply		High K supply	
Droughty	Irrigated	Droughty	Irrigated

Five hills of the following seed stocks were planted in each section: Irish Cobbler, Green Mountain, Katahdin, certified Rural New Yorker, and a stock of Rural New Yorker selected by test as darkening after boiling. The size of bed permitted planting distances of 1.5 feet between rows and 1.0 foot between hills. This density of stand appeared to be justified by the limiting light intensity of the culture period. Plants from the abnormal Rural New Yorker seed stock appeared about 3 weeks later than those from the other stocks. Restriction of water supply on the designated areas was begun about a month before harvesting.

The yields, on the basis of 3-foot spacing between hills and rows, ranged approximately from 16 to 160 bushels per acre. Low supplies of both water and potassium produced tubers with peaked ends, but this effect was noticeable also where the potassium supply was restricted and the water supply liberal. Slenderness of potatoes in relation to potassium deficiency has been observed by Martin et al. (*4*). The crop was stored at 50° to 60° F. for 1 month before it was tested for color after boiling. Light grayness was confined to tubers produced by the Green Mountain and normal Rural New Yorker stocks in the droughty area on the low-potassium treatment. No correlation was found between grayness and shape of tuber. The crop from discoloring seed stock was free from grayness.

SEASON OF 1936

Differentials of water and potassium similar to those of 1935 were supplied. Since there was an accumulation of potassium in the low potassium areas the used soil was replaced by a mixture similar to that used in 1935. This contained 125 and 75 pounds, respectively, of available K and P per acre, and had a pH value of 7.0. Its water-holding capacity was 27 percent. The application of complete fertilizer was equivalent to 1,200 pounds of 4-10-12 per acre, with 60 percent of the nitrogen in organic form. There was one-half as much available potassium in the control plot and no use was made of sodium as a substitute. After mid-March water was withheld from parts of the bed, and on April 6 the soil of the droughty and irrigated sections contained 2.1 and 8.0 percent (dry-weight basis), respectively; these values correspond to about 8 and 30 percent of saturation.

The potatoes were stored at 50° to 60° F. for about 1 month before boiling. The only samples that discolored were of the Burbank variety produced on the low-potassium areas, both under irrigation and exposed to drought. The range in yield was the same as in 1935. No correlation was noted between yield and degree of darkening after cooking; that is, there was no evidence of depletion of nutrients by high yields attended by tuber discoloration.

SEASON OF 1937

The primary purpose of the 1937 planting was to test the capacity of certain rarer mineral elements to prevent discoloration of potatoes produced with a relatively low supply of potassium. Burbank stock, which had been propagated at Hancock from high-grade seed stock obtained from Idaho in 1935, was used.

The residual soils in the bed sections now contained 125 to 150 pounds of available phosphorus per acre. Adjustments of the potassium content were made to give 150 pounds per acre in two of the sections and 275 pounds in the others. The pH was about 7.8 and the nitrate content was low. Two weeks after transplanting with seed pieces attached, two sections at each level of potassium supply were given an application of hydrated manganous chloride and boric acid at rates of 20 and 10 pounds per acre, respectively. Ammonium salt was applied from time to time until it reached a total of 80 pounds per acre. Shortening of the day was begun March 1 and 3 weeks later there was some fading of crown leaves, although tip growth was vigorous. This effect was especially noticeable on the low-potassium areas and may have resulted from subnormal utilization of nitrogen.

The crop yield was as high as 250 bushels per acre. The tubers were stored for about a month at 60° F. before they were tested, and many of the mature ones had previously been exposed to relatively high temperatures in the bed. Because of these conditions it was believed that the cooking test would give significant results. The results are shown in table 4.

It is worthy of note that tubers of the Burbank variety from all cultural treatments discolored when boiled while all tubers of the Chippewa variety cooked white. The Irish Cobbler discolored more generally than the Rural New Yorker, and developed on the low-potassium supply supplemented by rarer elements the localized intensity of discoloration in the stem end or cortical region which is characteristic of the most seriously affected potatoes. This experiment gives no consistent indication of cause and effect between fertilizer treatments and postcooking pigmentation of the susceptible varieties.

TABLE 4.—*Effect of a low supply of potassium with rarer elements added on yields and on discoloration of potatoes grown in a greenhouse bed, 1937, and effect of heat and drought with rarer elements limited or ample, 1938*

SEASON OF 1937

Variety	Water	Potassium	Rarer elements	Weight of fresh tubers	Boiling record [1]
				Grams	
Burbank	Ample	Low	None	781	LG
			Manganese and boron	778	MG
		High	None	1,510	LG
			Manganese and boron	1,219	LG
Chippewa	do	Low	None	722	W
			Manganese and boron	814	W
		High	None	1,405	W
			Manganese and boron	1,241	W
Irish Cobbler	do	Low	None	620	LG
			Manganese and boron	536	MG
		High	None	888	LG
			Manganese and boron	1,006	W
Rural New Yorker	do	Low	None	132	W
			Manganese and boron	207	W
		High	None	637	LG
			Manganese and boron	423	LG

SEASON OF 1938

		Soil heating during tuberization	Boron, copper, manganese, and zinc		
Chippewa	Restricted late	Applied	Limited	220	W
			Ample	49	W
		None	Limited	172	W
			Ample	171	W
Irish Cobbler	do	Applied	Limited	193	LG
			Ample	98	W
		None	Limited	131	LG
			Ample	167	LG
Rural New Yorker	do	Applied	Limited	21	W
			Ample	37	LG
		None	Limited	16	W
			Ample	36	W
Triumph	do	Applied	Limited	54	W
			Ample	119	W
		None	Limited	22	W
			Ample	58	LG

[1] W=white; LG=light gray; MG=medium gray.

SEASON OF 1938

In view of the failure to induce darkening after cooking consistently by fertility deficiencies and drought in the bed cultures of previous years, attention was directed in 1938 to another climatic factor, heat. An electric heating cable was installed [5] in one-half of the bed at a depth of about 3 inches from the surface of the soil. The unit was so distributed as to fall between hills in the rows, its operation being controlled by the combined use of a time switch on the electric service and a thermostat with its expansion element buried in the soil.

An examination of the residual soil showed that the soil reaction was uniform (pH 7.5) throughout the bed sections, as was also the distribution of ammoniacal nitrogen (5 pounds per acre). Dicalcium phosphate and sodium nitrate were added in quantities to give uniform values of 50 pounds per acre of nitrate and 85 pounds per acre of available phosphorus. The supply of available K was brought to 125 pounds per acre by the addition of potassium sulphate. It was assumed that the sections treated with manganese and boron in 1937 would retain sufficient residues of these elements; applications of 5 pounds per acre each of the hydrated sulfates of copper and zinc were added to these sections. Several weeks after planting, an additional 50 pounds per acre of ammonium salt was applied to all sections. The seed pieces were cut to approximate a 1-inch cube about each eye. When transplanted to the bed the Rural New Yorker had a very poorly developed root system.

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[5] Courtesies extended by representatives of the General Eelectric Co. in connection with the installation are appreciatively acknowledged.

The heater was put into operation from 8 a. m. to 4 p. m. on April 1, when the use of the lamps for controlling length of day was discontinued. The use of the heater was limited to 2 weeks because of the rapid maturity of the plant tops. On days when the outdoor radiation was about one-half to two-thirds normal the temperature differential between heated and unheated portions of the bed 3 inches below the surface was about 7° F. at midday, or an interval of 86° to 79° F. Immediately after the heater was turned on the soil of the entire bed became dry. Determinations on April 9 showed the water content in the heated portion of the bed to be 2.4 percent where boron, copper, managnese, and zinc were limited and 2.6 percent where these elements were ample; in the part of the bed where heat was not applied the water content was 2.7 percent where these elements were limited and 4.9 percent where they were ample. It may be supposed that sheltering from direct sunlight by the southern end wall of the house contributed to a greater retention of water in the last section. Table 3 shows that the season was unusually cloudy.

The boiling test was made after the tubers had been stored for 1 month at about 60° F. The test showed that heat and drought, operating simultaneously, and primarily on the developing tuber, had no detectable influence upon darkening after cooking (table 4). However, the vines were made somewhat independent of drought by the practice of frequently sprinkling the unoccupied floor of the house. In this experiment Irish Cobbler was most generally subject to discoloration while Chippewa remained white throughout the tests. There is no evidence that the application of rarer elements was beneficial so far as whiteness of the cooked tubers was concerned,

POT CULTURES

Pot culture was adopted primarily for the purpose of isolating the plants from influences of the subsoil in the greenhouse bed. The containers most frequently used were galvanized-iron boxes, 1 foot square and 8 inches deep, adapted to the use of 50 pounds of sand. To protect against contamination, the inside of the boxes was coated with paraffin. Ottawa silica sand, screened to 30–40 mesh and analyzing about 99.5 percent SiO_2, served as the culture medium.

In terms of commercial fertilizer, the regular nutrient mixture supplied approximated 1,000 pounds per acre of a formula which ranged from 6.5–15–15 in 1936 to 2–18–23 in 1938. Appropriate amounts of the minor nutrient elements were added in various combinations to some cultures. The proportions of salts used are shown in table 5, and the variations in the supply of potassium and boron are given in the text. An application of 1.0 gm. per pot approximates the equiv-

TABLE 5.—*Nutrient applications to pot cultures* [1]

Salt applied	1936	1937	1938	1939
	Gram	*Gram*	*Gram*	*Gram*
Dicalcium phosphate, hydrated	5.0	5.0	5.0	5.0
Magnesium nitrate, hydrated	5.0	3.0	3.0	3.0
Potassium sulfate	4.0	4.0	5.0	4.0
Ammonium sulfate	[2] 3.0	2.4	1.0	1.4
Ferric citrate	.3	.3	.3	.3
Manganous sulfate, hydrated	.03	.025	.025	.025
Boric acid	[3] [4] .0006	.0005	.030	.030
Copper sulfate, hydrated	[4] .0003	.005	.030	.005
Zinc sulfate, hydrated	[4] .0003	None	.015	.005

[1] See text for departures from normal.
[2] Ammonium chloride used instead of ammonium sulfate.
[3] Borax used instead of boric acid.
[4] Estimated amount.

alent of 100 pounds per acre, on the area basis. Phosphorus and iron carriers were added before transplanting and the rarer mineral elements and part of the magnesium salt were added immediately after transplanting. The remainder of the major elements were added as the plants developed. With the shift of proportions between calcium and magnesium salts the proportion between molecular equivalents of calcium and magnesium oxides was increased from about 2.0:1.0 to 3.5:1.0.

The water content of the soil was controlled by weighing the containers and contents when watering appeared necessary. In this way wide differences were maintained in the water content of different cultures. The plants in the pot experiments were subjected to the same use of artificial illumination as those in the beds, but they were not covered for shortening the day length as maturation approached.

SEASON OF 1936

In the 1936 experiments the salt applications were varied to provide different levels of potassium carriers (2, 4, and 6 gm.), and these were associated with similar variations in the accompanying sulfur supply. A separate group of cultures of Rural New Yorker received liberal proportions of calcium with the highest level of potassium. The calcium was supplied in two forms, one (hydrated calcium sulfate, 6.8 gm. per pot) to provide for mass effects in its proportion to potassium and other elements and the other (calcium carbonate, 8.0 gm. per pot) to simulate the condition of reduced availability of other mineral elements brought about by change of reaction in the liming of soil. The pH of the culture medium, as determined during the late vegetative activity of the plants, seemed too little influenced by the form of calcium supplied to be an important factor in the availability of mineral elements. Leachings of the medium to which calcium carbonate was added had a pH of 6.3, and those from the cultures supplied calcium sulfate had a pH of 5.9, as determined by the colorimetric method.

One month after transplanting marked differences were apparent in the development of the tops of different plants and between some treatments of the Rural New Yorker. The plants not given the extra application of calcium salts had rather slender stems, those receiving calcium sulfate were short and much branched, while those receiving calcium carbonate were both tall and rugged. Terminal buds of plants in the group treated with calcium sulfate showed desiccation and fading in a manner indicative of toxicity from excess calcium. By the middle of March yellowing of the basal leaves in many cultures had become conspicuous, but this effect showed no correlation with low supplies of potassium. The addition of the third gram of nitrogen carrier at that time was followed by partial recovery.

By April the leaves of the low-potassium cultures of Rural New Yorker showed a tendency to roll, but the severity of the leaf roll decreased as the supply of potassium increased (fig. 1). Although the effects of potassium deficiency were less severe on Triumph than on Rural New Yorker, the former variety suffered some bleaching of the lower leaves. This latter effect was prominent in the Chippewa variety, which developed the least rolling of leaves. The symptoms produced on the leaves in this experiment closely resemble those found by Jones and Brown (*3*, *p. 123*, *pl. 5 B*) and others to be caused by a deficiency of boron. If so produced, they must represent a relatively mild deficiency, as death of the main stem tips and regeneration from lower branches, which will later be shown to attend an extreme lack of boron, were not observed. Only a limited application of boron was made directly (approximately 0.0002 pound per acre), and any additional supplies were provided by water, sand, and incidental sources. According to the findings of Carson,[6] the total boron added per culture in such indirect ways may have been at least as much as 0.0006 pound per acre. The improvement in appearance of leaves as the application of potassium was increased might have been due to

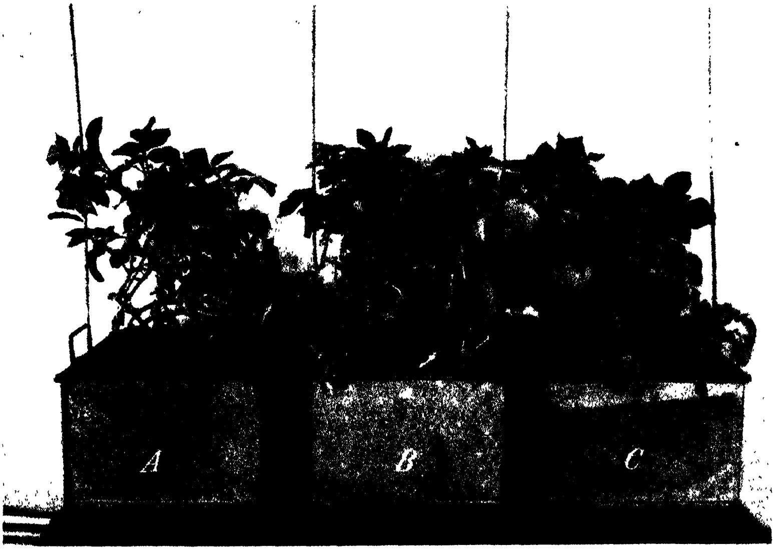

FIGURE 1.—Plants of the Rural New Yorker variety of potato, grown at different levels of potassium supply, showing symptoms of boron deficiency: *A*, 200 pounds; *B*, 400 pounds; *C*, 600 pounds of K_2SO_4 per acre, 1936.

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[6] CARSON, R. B. AN INVESTIGATION OF BORON IN RELATION TO POTATO DISCOLORATION. (Unpublished this is filed in the University of Wisconsin library.) 1939.

contamination of its salt with boron. Analysis of the writers' stock K_2SO_4 showed a content of 6.7 p. p. m. of boron, which would give a boron equivalent of 0.0034 pound per acre for the greatest application of this salt. This is about four times the amount accounted for above.

The crop was harvested on April 26 and tested May 8, which was probably too soon for the development of the post-harvest changes involved in the full expression of discoloration after boiling. The yields ranged from 320 to 635 gm. per pot, equivalent to 170 to 340 bushels per acre on the hill basis for clay loam. With the exception of the Rural New Yorker cultures to which calcium carbonate was supplied, none of the samples darkened appreciably after cooking, and even the Rural New Yorker would not have appeared discolored after peeling, as was subsequently discovered.

SEASON OF 1937

In the 1937 experiments emphasis was placed primarily on variation in the combination of the rarer mineral elements, superimposed upon a low (2.0 gm.) supply of potassium carrier. The combinations of the mineral nutrients are shown in table 6.

TABLE 6.—*Effect of various combinations of the rarer elements with a low supply of potassium on discoloration of potatoes grown in pots of sand or soil in the greenhouse, 1937*

Variety	Culture medium	Potassium supply	Rarer elements added [1]	Weight of fresh tubers [2]	Boiling record [3]
				Grams	
Chippewa	Sand	Low	Boron and manganese	435	W, W.
			Boron and copper	341	W, LG.
			Copper and manganese	118	W, W.
			Boron, copper, manganese.	337	W, W.
			None	110	W, W.
		Medium	do	92	W, W.
Rural New Yorker	do	Low	Boron and manganese	441	MG, W, W.
			Boron and copper	465	LG, W, W.
			Copper and manganese	172	W, W, W.
			Boron, copper, manganese.	354	W, LG, LG.
			None	140	W, W.
		Medium	do	117	LG, MG, W.
Burbank	Antigo clay loam		do	571	W, W.
			Boron	551	W, W.[4]
			Manganese	589	W, W.
			Boron and manganese	497	W, W.[4]
Irish Cobbler	Plainfield sandy loam.		None	387	W, W.[4]
			Boron	317	W, LG.
			Manganese	365	W, W.[4]
			Boron and manganese	315	W, W.[4]

[1] On Antigo clay loam and Plainfield sandy loam rare elements were applied with common nutrients.
[2] Total of duplicate cultures.
[3] Record of individual cultures: W=white; LG=light gray; MG=medium gray.
[4] Smallest tubers boiled light gray.

Soils from the northern and central (sandy) potato-producing areas were also planted to the Irish Cobbler and Burbank varieties respectively. The former had cooked white but was uncertified; the latter has been described in connection with the bed culture of 1937. Each of these soil cultures received 1.0 gm. of ammonium sulfate and monocalcium phosphate, 2.0 gm. of potassium sulfate, and either 0.5 gm. of hydrated manganous sulfate or 0.1 gm. of boric acid, or both.

Drying of the stem tips occurred in both varieties on sand, in association with lack of boron. There was also, as in the results of the previous year, a protective effect where potassium was liberally supplied to Rural New Yorker, as shown in figure 2. This effect might again be ascribed to an increase in boron. With the Chippewa there was serious bleaching of the main stem tips but relatively vigorous basal replacement growth. Both varieties developed essentially normally on the low level of potassium supplemented by either boron and copper or boron and manganese.

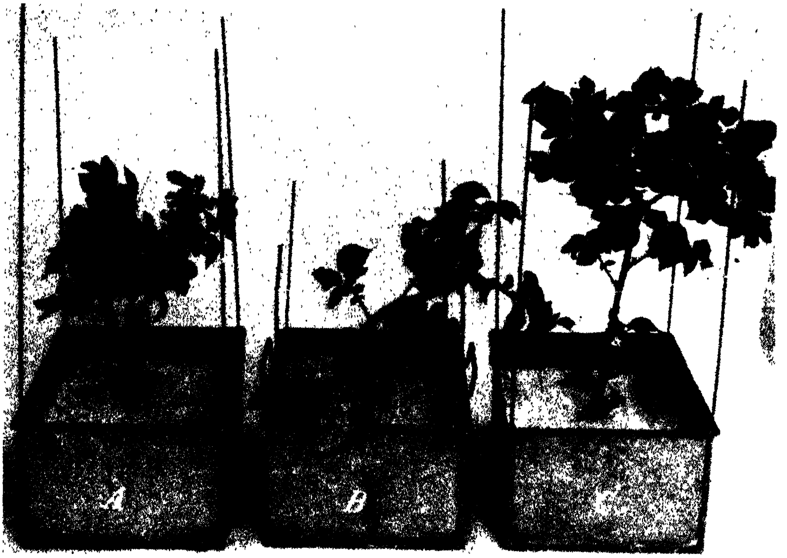

FIGURE 2.—Plants of the Rural New Yorker variety of potato, given a liberal supply of potassium, showing the protective effect of boron: *A*, 400 pounds of K_2SO_4 per acre; *B*, 200 pounds of K_2SO_4 per acre; *C*, 200 pounds of K_2SO_4 plus B and Mn per acre. 1937.

The yields of the sand cultures show clearly the significance of boron in relation to the weight of tubers produced. As the boiling test was made about 4 weeks after harvesting, the results should have significance. The Chippewa variety was practically free of darkening after cooking while the Rural New Yorker was rather generally subject to this abnormality. The irregularity of discoloration of the Rural New Yorker variety in some treatments suggests variation in the character of the mother tuber as a possible cause. This seems a more probable cause of the irregularity than differences in the culture medium from pot to pot. Unless a questionable emphasis is placed upon the grayness of very small tubers, the soil cultures could be considered relatively free from discoloration.

SEASON OF 1938

In 1938 some of the plants were raised in glazed earthenware crocks holding 33 pounds of the silica sand. Various combinations of the rarer mineral elements were supplied as supplements to a lower level

(3 gm. of salt) of potassium as shown in table 7. A variation in iron supply was introduced by adding 7.5 times the usual amount of carrier with the fullest complement of rarer elements on the lower level of potassium. In the box cultures boric acid was applied in equivalents of 0.03, 0.3, and 3.0 pounds per acre, on each level of potassium.

TABLE 7.—*Yield and boiling records of potatoes produced with different combinations of the rarer elements and with different proportions between potassium and boron in greenhouse cultures, 1938*

POT CULTURE, ALL ON LOW POTASSIUM SUPPLY

Variety	Rare element added	Weight of fresh tubers[1]	Boiling record[2]
		Grams	
Irish Cobbler	Manganese, copper, zinc	112	W, W Sp sp
	Manganese, boron, zinc	197	W, W
	Manganese, boron, copper	170	W, W
	Manganese, boron, copper, zinc	156	W, W SP
	Boron, copper, zinc	193	W, W sp
	Manganese, boron, copper, zinc, high iron	172	W, W
Rural New Yorker	Manganese, copper, zinc	31	LG, LG SP, SP
	Manganese, boron, zinc	54	W, LG SP
	Manganese, boron, copper	36	LG, W
	Manganese, boron, copper, zinc	27	LG, W sp
	Boron, copper, zinc	[3] 30	W sp
	Manganese, boron, copper, zinc, high iron	[3] 45	LG

BOX CULTURE AT DIFFERENT K AND B LEVELS

Variety	Level of K supply	Level of B supply	Weight of fresh tubers[1]	Boiling record[2]
Chippewa	Low	Low	247	W, W
		Medium	202	W, W
		High	264	W, W
	High	Low	158	W, W
		Medium	160	W, W
		High	174	W, W
Rural New Yorker	Low	Low	36	SP W, W
		Medium	102	W, W
		High	71	W, LG
	High	Low	[3] 17	LG
		Medium	53	LG, LG SP sp
		High		

[1] Sum of duplicate cultures except as noted.
[2] Record of individual cultures: W = white; LG = lighr gray; SP = spraing extensive ; sp = spraing limited.
[3] No tubers in 1 culture.

To minimize mineral contribution by the seed pieces, these were cut in the form of about 1-inch cubes. Transplanting of the Rural New Yorker stock was delayed 10 days because it was found deficient in root development. Although, like the other varieties, it was certified seed stock, its behavior in sprouting suggested that it was not normal.

Disturbances in vegetative development, which were first observed on March 20, were consistently related to cultural treatment. The pot cultures which received no boron showed withering and darkening of the tips of main stems and the leaves adjacent. Five days later the larger crown leaves were fading perceptibly, especially on the

Chippewa plants. This checking effect upon growth was followed by accelerated growth of the basal branches, which became conspicuous by March 30. In other cultures on the same level of potassium supply, marginal "burning" of leaves was associated with an application of only 3 pounds of boric acid per acre. Cultures not supplied zinc developed clusters of undersized youngest leaves in the Rural New Yorker variety, but this abnormality was not apparent in the Irish Cobbler. The high application of iron carrier was associated with abnormal upper leaves in the Rural New Yorker, these leaves being somewhat faded, brown at the margins, and rather stiff.

No anatomical abnormality of the conducting system, such as occurs in root nodules of leguminous species deprived of boron (*8*), was found in discoloring tubers.[7]

The Rural New Yorker produced small tops in the sand cultures in association with initial weakness of roots. On the used soils, replenished in major nutrients, they were thrifty but produced very small tubers.

The records of these crops are assembled in table 7. The potatoes stood for 1 month in an unrefrigerated storage room before they were boiled. The Irish Cobbler cooked a normal white, but in the raw state suffered from a form of internal brown spotting known as "spraing," or internal rust spot. The Rural New Yorker discolored more generally after cooking and was more subject to spraing. In both varieties the association of spraing with the boronfree mixture of nutrients was somewhat conspicuous. The high plane of iron salt in some of the cultures does not seem to have affected either the yield or the discoloration of tubers.

There was no discoloration of tubers of the Chippewa variety grown in boxes with different combinations of potassium and boron. On the other hand, several of the Rural New Yorker cultures in this series produced discoloring tubers, although without consistent relation to nutritional treatments. This variety also developed spraing, but again without any apparent relation to the nutrient supplied. There seems to be some correlation between soil-moisture content and the appearance of this form of internal browning. After March 20 the level of moisture at watering was decreased from 12 to 5 percent of the weight of dry sand, or essentially from 50 to 20 percent of saturation. Later the house became hot and the cultures lost water rapidly. These were desirable circumstances, as they closely simulate the weather conditions that aggravate discloration of the field crop after cooking. The water content of the sand in some of the crocks was found to be less than 3.0 percent. The severity of spraing developed in the Rural New Yorker variety under these conditions is shown in figure 3. It should be recognized that the driest cultures probably suffered the greatest elevation of temperature. This factor might either act independently or aggravate the effects of drought. From the irregularity of distribution of both darkening after boiling and spraing, it seems likely that extremes of drought and heat in some cultures contributed to these abnormalities. However, as suggested for previous crops, it is advisable to recognize peculiarities of the mother tuber as a possible factor in these responses.

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[7] Examination was made by Elaine Tottingham under the direction of Prof. Emma L. Fisk of the Department of Botany.

SEASON OF 1939

The 1939 studies were devoted primarily to the role of seed stock in discoloration after cooking. It is not to be assumed that disease is the cause of discoloration, for in several examinations of commercial plantings the writers have found no association of blackening with symptoms either of yellow dwarf or leaf roll in the parent plant. Moreover, some recent results indicate that poor fertility of the soil and unfavorable weather conditions can induce postcooking discoloration of tubers from Rural New Yorker stock that is apparently normal.

Cultures in the iron boxes were exposed to heat when the development of the tubers was well under way. This was done by placing the boxes over the heating cable previously used in the bed planting. The maximum variation between heated and control cultures at midday in early April ranged from about 31° to 23° C. (88° to 73° F.), or

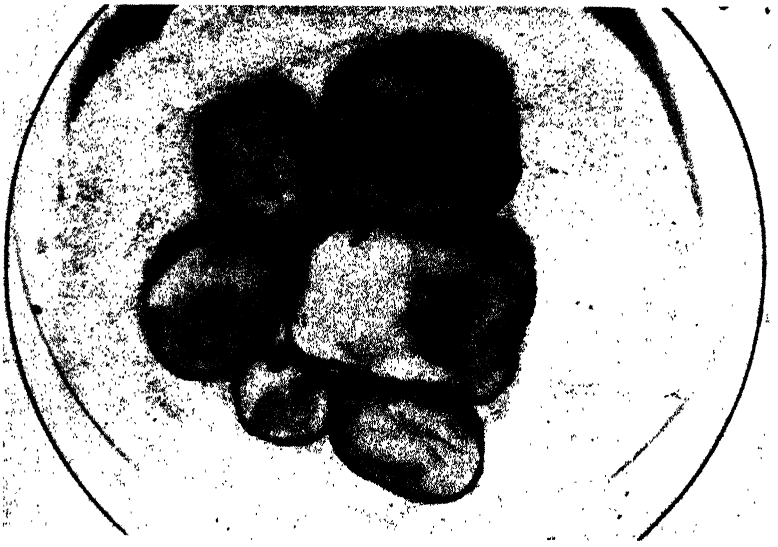

FIGURE 3.—Potatoes of the Rural New Yorker variety, grown in sand in which the moisture content fell below 3.0 percent, showing development of "spraing," or internal browning, 1938.

an interval of 8° C. (15° F.). By way of furnishing more favorable conditions for tuber development, cylindrical iron pots holding 63 pounds of sand were substituted for the smaller iron boxes in some of the experiments. Like the metal boxes these were coated on the inside with paraffin.

For the high-potassium level the fertilizer application in box cultures was equivalent to about 1,000 pounds of 3–20–23 per acre in field practice. One-half as much potassium was provided on the low level. All nutrients were increased 25 percent in the pot cultures commensurate with their increased size. The rarer nutrients were added in the amounts shown in table 5, except for the omissions of boron specified in table 8. These amounts appear small as compared with those supplied in field practice, but since they remain fully available, they should be adequate. The primary consideration was to avoid toxicity resulting from an excess of these elements. The boron supply was added also at levels of 0.2 and 0.02 of the normal.

The seed stocks planted included tuber-test selections for either whiteness or discoloration after cooking and the presence of spraing. About 1 month after transplanting, the plants from discoloring seed stock were slender-stemmed and basally defoliated, and the crown leaves were small and crinkled. An examination of these plants by Dr. Russell Larson of the Department of Plant Pathology disclosed no symptoms of virus disease. Moreover, short growing tests in flats conducted under his guidance failed to reveal any such abnormality. About 10 weeks after planting, boron deficiency was partly compensated by the addition of 2.0 mg. of boric acid per culture, to aid tuber production in cultures showing injury from its omission. Three days later, selected cultures were restricted as to water supply, and these formed the subseries exposed to droughty conditions. The heating cable was operated from April 1 to 17. By April 3 some of the cultures in the heated group showed the desiccation of main stem tips characteristic of boron deficiency. Records of yield and discoloration are given in table 8.

TABLE 8.—*Yields and boiling records of potatoes grown in the greenhouse at different heat, moisture, and nutrition levels, showing influence of the seed stock on discoloration and the presence of spraing, 1939*

Seed stock	Soil moisture	Maximum soil temperature		Boron added	Weight of fresh tubers [1]	Boiling record [2]
	Percent	*°C.*	*°F.*		*Grams*	
Normal: Rural New Yorker A.	60	31	88	Yes	332	W, W, W, LG.
				No	300	W, W, W, W.
		23	73	Yes	254	W, W, W, W.
				No	283	W, W, W, W.
	20	31	88	Yes	317	W, W, W, LG.
				No	269	W, W, W, W.
		23	73	Yes	332	LG, LG, LG, W.
				No	223	W, W, W, W.
Discoloring: Rural New Yorker B.	60	23	73	Yes	128	W, LG.
	20	31	88	do	194	LG, LG.
Affected by spraing: Rural New Yorker B.	60	23	73	do	164	MG, LG. SP.
	20	31	88	do	142	MG, LG. SP.
	Variation in nutrients		Potassium supply			
Apparently normal: Rural New Yorker D.			Low	None	53	W, W.
				Low	119	W sp, MG.
				Medium	141	LG, MG.
				High	133	LG, W.
			High	None	134	W, W.
				Low	104	W, W.
				Medium	220	W, W.
				High	112	MG, W.
Discoloring: Rural New Yorker B with spraing.	High N(NH_4)				196	LG, LG, LG. sp sp.
	Mixed N (NH_4 and NO_3)				219	LG, MG, MG. Sp sp sp.
	Low P				198	LG, LG, MG. Sp sp sp.
	Medium P				279	W, LG, LG. Sp sp.

[1] Sum of cultures recorded in boiling record.

[2] Record of individual cultures: W=white; LG=light gray; MG=medium gray; SP=heavy spraing sp=light spraing.

An examination of the results from heat and drought (table 8) shows little discoloration of the crop from normal Rural New Yorker seed stock. The most definite off color which occurred was under the combination of low soil temperature and low soil moisture, with boron added. This latter circumstance further indicates that blackening of cooked tubers is not due to lack of boron. It is worthy of note that spraing appeared to a limited extent under the particular combination of cultural conditions that was most definitely associated with darkening after cooking (table 8). In the present state of knowledge (*2*) it may be assumed that the phenol oxidases were unduly active in darkening tubers, with perhaps also an increased tissue content of either simpler phenolic compounds or tannins.

The irregular occurrence of discoloration within a given cultural treatment (temperature—moisture and potassium—boron series, table 8) is suggestive of individual variation either of environmental conditions in the pots or of a tendency to discolor transmitted by the mother tuber. In view of the close control of environmental factors, the results seem to indicate that the condition of the tuber planted is a primary factor in the development of blackening after boiling. Discoloration was rare and apparently independent of fertilizer additions in the series of crops grown from normal seed stock. On the other hand, crops grown from discoloring seed stock discolored in practically every instance. The use of nitrate in considerable quantities was attended with more serious discoloration than the use of ammonium salt only. However, in more recent work nitrogen has been supplied only as nitrate without resulting in the production of discoloring tubers.

Since potatoes grown under glass differ in respect to light and other environmental factors from those grown in the field, the results obtained in these greenhouse studies may not be entirely comparable to results obtained under field conditions. The nature of the metabolic disturbances associated with the blackening of potatoes after cooking has been covered by earlier papers from this laboratory (*7*, *5*, *2*). Apparently some related significance should also be given to the concept of Szent-Györgyi (*6*) that disease as well as mechanical injury conduce to the formation of melanin.

SUMMARY

A series of greenhouse studies has been conducted over a period of years to determine the cause of darkening in potatoes after boiling.

Different varieties of potatoes were grown on sandy soil in bed and pot cultures in the greenhouse during the winter and early spring. Different combinations of mineral nutrient elements were applied at different levels to the soil and to pure sand. Some of the plantings were subjected to differences in water content and temperature of the culture medium. The mature tubers produced were tested for discoloration after boiling, usually after unrefrigerated storage for 1 month.

The blackening of these crops after boiling depended most directly upon the record of the tubers planted. In agreement with observations on commercial crops, this abnormality appeared also primarily as a varietal characteristic. Discoloration was common in the varieties Rural New Yorker and Irish Cobbler but rare in Chippewa and Triumph.

Differences in the rates of supply of the major nutrient elements and of iron and boron did not affect darkening. Neither did the omission of manganese, copper, and zinc, other than as accidental constituents. Deficiencies of boron which brought about growth disturbances ranging from leaf roll to break-down of stem tips did not induce discoloration of boiled tubers.

Subjecting the developing tubers to heat, drought, or a combination of these factors, did not cause a consistent discoloration of the cooked tuber. "Spraing," or internal brown spotting, occurred in tubers subjected to less than 3.0 percent moisture in the sand, but this abnormality was not universally associated with blackening after boiling.

The stocks which produced discoloring crops were apparently free from the common potato diseases. However, since the tendency to discolor is inherited, an unrecognized virus or other disease may be present in such stocks.

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expressed by 21 x^2. Thus the class interval increased as a function of the class number squared. The resulting segregation in the F_2 and the variation of the parent plants are given in table 10.

TABLE 10.—*Frequency distribution of plants according to hydrocyanic acid content*

Cross No.	Parents and F_2 progeny	Distribution by class interval (upper limit) according to hydrocyanic acid content in p. p. m.										Mean hydrocyanic acid content
		21	84	189	336	525	756	1,029	1,344	1,701	2,100	
		No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*P.p.m.*
1	Parent 1	0	3	5								118
	Parent 2	0	0	0	0	1	7	1	1			669
	F_2	0	0	23	26	18	4					286
2	Parent 1	4	4	1								40
	Parent 2	0	0	0	0	5	3	1				530
	F_2	2	6	27	26	16	7	4	1			296
3	Parent 1	4	4	2								50
	Parent 2	0	0	0	0	1	7	1	1			669
	F_2	0	4	9	29	24	20	4	2			464
4	Parent 1	4	4	2								50
	Parent 2	0	0	0	0	6	3	1				553
	F_2	0	3	5	18	32	30	9	2			445
5	Parent 1	0	16	4								60
	Parent 2	0	0	0	0	6	3	1				530
	F_2	0	1	3	9	27	30	11	4			549
6	Parent 1	0	2	3	5							175
	Parent 2	0	0	0	0	6	3	1				519
	F_2	0	5	8	16	24	9	8	3			421
7	Parent 1	0	4	1	4							190
	Parent 2	0	0	0	0	1	2					566
	F_2	0	1	9	27	25	16	3				283
8	Parent 1	0	4	2	3	1						185
	Parent 2	0	0	0	0	6	3	1				519
	F_2	0	0	2	14	15	9	9				644
9	Parent 1	0	1	5	1	3						257
	Parent 2	0	0	0	0	1	2					566
	F_2	0	1	7	24	32	21	7	1			435
10	Parent 1	1	4	5								101
	Parent 2	0	0	0	0	0	0	2	3	2	1	1,267
	F_2	0	1	5	3	10	13	14	19	9	4	896
11	Parent 1	3	4	3								70
	Parent 2	0	0	0	0	0	0	2	3	2	1	1,267
	F_2	0	8	10	14	17	15	11	4	1		475
Total	Parent 1	16	50	33	13	4						
	Parent 2	0	0	0	0	33	33	11	8	4	2	
	F_2	2	30	108	206	240	174	80	36	10	4	

The data appearing in table 10 indicate that the distribution of the F_2 populations on the basis of their hydrocyanic acid content in most cases approaches the limits of both parents. In some populations the distribution reaches the extremes of both parents. In 5 of the 11 populations, the lower limit of the parent plant low in hydrocyanic acid is not reached by the F_2 distribution, while in 5 cases the upper limit of the high hydrocyanic acid parent was surpassed. Individual F_2 populations show considerable variation in distribution. The total F_2 distribution approaches the normal curve. These data indicate that the level of hydrocyanic acid is definitely inherited. It also appears from the F_1 and F_2 data that dominance is lacking. Nevertheless, the tendency for the F_2 distributions to be skewed to the right indicates that the genes concerned do not act in strictly additive fashion. From both breeding observations and F_2 data, there is evidence that more than a single pair of genes are involved in the production of hydrocyanic acid in Sudan grass. The exact number cannot be estimated from these data.

Crosses involving parents which were low in hydrocyanic acid were made to determine the effect of hybridity on the level of hydrocyanic acid in the F_2. The distribution of hydrocyanic acid in parts per million of the parents and F_2 populations of four crosses between parents low in hydrocyanic acid is reviewed in the histogram shown as figure 1. The data indicate that the hydrocyanic acid content of the F_2 plants was essentially the same as that of the parents. Three plants appeared with a hydrocyanic acid content somewhat higher than that of the parents, but there was no general tendency for the F_2 to be higher in hydrocyanic acid than the parents. The increased vigor of the F_1 and F_2 plants was not accompanied by an increase in hydrocyanic acid content. The results appear to indicate that vigor lost on selfing may be restored by hybridization without increasing the hydrocyanic acid content. However, further investigations including plants which are more variable in their hydrocyanic acid content than those used in these studies will be necessary before any generalization can be made relative to the effect of hybridization on the hydrocyanic acid content.

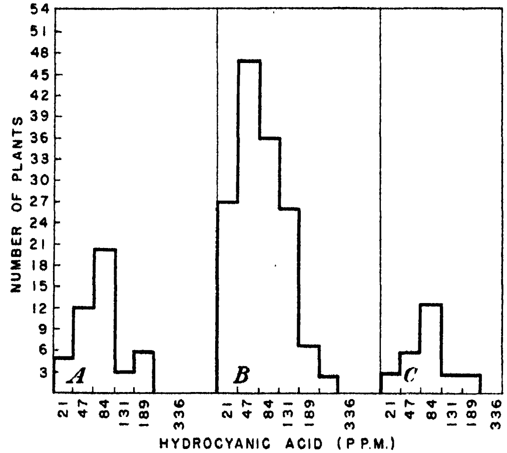

FIGURE 1.—Distribution of parent and F_2 populations of four crosses between plants low in hydrocyanic acid, based on hydrocyanic acid content: *A*, Parent population; *B*, F_2 population; *C*, parent population.

SUMMARY

The purpose of this investigation was (1) to determine the nature of the inheritance of the cyanogenetic glucoside durrin, (2) to develop strains of Sudan grass (*Sorghum vulgare var. sudanene*), so low in this glucoside as to materially reduce or eliminate entirely the danger of poisoning to livestock, and (3) to determine the effect of various environmental factors on the hydrocyanic acid content of the plant so that due consideration could be given them in the studies on inheritance and inbreeding.

Both the Boyd method and the method of Nowosad and MacVicar were found to be sufficiently rapid, accurate, and applicable to the conditions imposed by the study to warrant their use in determining the hydrocyanic acid content of bulk lots and single plants of Sudan grass.

The second growth of Sudan grass contained approximately twice as much hydrocyanic acid as the first growth.

The hydrocyanic acid content of Sudan grass increased to some extent as the moisture content of the soil decreased. No variation in hydrocyanic acid attributable to the diurnal factor was observed. A highly significant positive correlation coefficient was found between temperature and the hydrocyanic acid content of the plants.

The hydrocyanic acid content of the plant tissue decreased as it became older. Meristematic parts of the plant were significantly higher in their content of hydrocyanic acid than the older parts of the plant. Young actively growing tillers were found to be uniformly and constantly high in hydrocyanic acid even though other parts of the plant were well advanced in growth. The hydrocyanic acid content of plants was determined by analyzing tillers 5 to 7 inches in height. By using young tillers it was possible to obtain comparable plant material throughout most of the growing period.

Flowering in Sudan grass was usually completed by 8 a. m. during bright, warm weather. The viability of the pollen was negligible 5 hours after it was shed. Sudan grass grown at Madison, Wis., was largely self-pollinated. Approximately 7 percent of natural crossing was found to occur.

The yield of forage and seed produced by 20 selected inbred lines was determined in 1939 and 1940. The average yield of dry forage produced by the inbred lines in 1939 and 1940 was 82.2 and 95.2 percent, respectively, that of commercial Sudan grass. The average yield of seed produced by the inbred lines in 1939 and 1940 was 75.3 and 61.8 percent, respectively, that of commercial Sudan grass.

One hundred and seventy-five inbred lines were tested for their hydrocyanic acid content in 1938, 1939, and 1940. The results indicate that an inbred line which is high in hydrocyanic acid in any given year will remain relatively high from year to year, while an inbred which is low in hydrocyanic acid in any given year will remain relatively low from year to year. No positive correlation was found to exist between the crude protein and the hydrocyanic acid content of the plant.

Vigorous strains of Sudan grass uniformly low in hydrocyanic acid were developed by hybridizing plants low in their content of hydrocyanic acid.

The hydrocyanic acid content of Sudan grass is not controlled by a single pair of genes. The hydrocyanic acid content of F_1 plants was intermediate between that of the parents. F_2 populations of plants from crosses between parents low in hydrocyanic acid were low in hydrocyanic acid. F_2 populations of plants from crosses between parents low and high in hydrocyanic acid resulted in a distribution extending to the limits of the parents with no tendency to be bimodal.

Vigor lost by selfing may be restored by hybridization without accompanying increases in hydrocyanic acid in the F_1.

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STAND IRREGULARITY AND ITS RELATION TO THE YIELDS OF SWEET CORN[1]

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INTRODUCTION

In the opinion of most canners and growers the yields of sweet corn are closely related to the uniformity of the field stand. Considerable care is taken to grade the seed into as many as 6 to 10 different sizes and to have available special planter plates for each seed size in order to assure a uniform drop. Having the proper equipment, seedsmen are often called upon to do the grading, a practice which they consider wasteful since buyers refuse to accept odd-sized grades and "round" kernels, which no planter plates will drop uniformly. Inasmuch as most of the sweet corn hybrids are single crosses it is virtually impossible to secure the uniformity of size and shape of seed associated with double-crossed field corn. Seedsmen's losses in grading, in addition to those associated with the regular cleaning process, frequently run as high as 10 percent, which, of course, adds considerably to the already high selling price.

It is self-evident that even with a uniform drop the field stand, because of natural hazards, will be more or less irregular. The tendency is to replant fields if the stands vary by more than one plant per hill on the assumption that this is absolutely necessary in order to secure maximum yields. The experiment herein reported was designed to determine the effect on yield of uniform stands as compared with irregular stands, including missing hills.

PLAN OF THE EXPERIMENT

The writer [2] has shown that under conditions at Urbana, Ill., the optimum planting distance for open-pollinated Country Gentleman sweet corn (*Zea mays*) is 40 by 40 inches with a stand of three plants per hill where weight is the primary consideration and four plants per hill where count is the important factor. Therefore, in the present experiment the planting distance of 40 by 40 inches in check rows was used with a maximum stand of four per hill.

The experiment was conducted at Urbana for the 3 years 1937–39 and included all the arithmetically possible stands between 0 and 4 per hill, allowing for a single variation in every other hill. There are 14 possible combinations, as follows:

0 and 1
0 and 2
0 and 3
0 and 4
1 and 1
1 and 2
1 and 3
1 and 4
2 and 2
2 and 3
2 and 4
3 and 3
3 and 4
4 and 4

Since including stands of 0–1 and 1–1 would probably add but little experimental information, the lay-out was limited to the remaining 12 planting rates, 3 of which (2–2, 3–3, and 4–4) were uniform.

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[1] Received for publication October 3, 1942.

[2] HUELSEN, W. A. YIELD OF SWEET CORN IN RELATION TO DISTANCE AND RATE OF PLANTING. Ill. Agr. Expt. Sta. Bul. 487, pp. 34–104, illus. 1942.

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The plots having missing hills or irregular stands were planted so that in the odd-numbered rows, including border rows, the odd-numbered hills contained either the missing hill or the lower number of plants per hill. In the even-numbered rows, on the other hand, the sequence was reversed. This procedure assured an equal number of hills within plots for each of the two planting rates or for the respective missing hills and planted hills in irregular stands. In addition, the pattern was identical in all plots.

An experiment of this kind with only 12 variables lends itself well to a predetermined statistical treatment, and it was therefore laid out on the basis of a Latin square. Snedecor[3] presents a plan for a Latin square with 12 variables, and this was followed exactly in the field during the 3 years the experiment was conducted. However, precautions were taken to move the experiment to a new site each year, so that soil variations would be equalized.

Individual plots were laid out 6 rows wide by 12 hills long with an alley surrounding each plot. The day before harvest the outside row on all 4 sides of each plot was cut with a corn knife and removed. Thus the net plot actually harvested consisted of 4 rows, each 10 hills long, a total of 40 hills, as compared with the gross plot of 72 hills (6 rows, 12 hills each). It was considered that essentially all border effect was eliminated by this procedure. The net plot was 0.0102 acre in size.

Every possible effort was made to have the actual survival of plants at harvest time coincide with the indicated rate for each plot. The plots were planted at least twice as thickly as required and the seedlings were thinned to the proper rate in each hill when 4 to 6 inches high according to the pattern described above.

Illinois Country Gentleman Cross 8 × 6 was used throughout. The seed was obtained each year from an Illinois canning company specializing in the growing of its own seed. The seed was graded to the same uniform size each year and treated with the same dust disinfectant for control of seed-borne seedling disease organisms.

Variations in maturity between plots were relatively slight, but these were taken care of by harvesting each plot 21 or 22 days after the appearance of the majority of the silks, according to season.

All the ears, including undeveloped shoots, provided they showed silks, were harvested from each plot at the proper time, sacked, and brought to the laboratory. The unhusked ears were sorted into usable ears and culls. The former were then husked by machine and sorted again into prime husked ears and husked culls. No dented ears were found as the harvesting was completed before denting occurred. The prime husked ears were cut and scraped with a standard cream style cutter. Suitable counts and weights were taken at the various stages and the grading practices were similar to those followed by commercial canners in Illinois.

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[3] SNEDECOR, G. W. CALCULATION AND INTERPRETATION OF ANALYSIS OF VARIANCE AND COVARIANCE. 96 pp. Ames, Iowa. 1934. (Iowa State Col., Div. Indus. Sci. Monog. 1.)

STATISTICAL METHODS

The methods of computing the annual yields were those mentioned by Snedecor[4] [5] for the variance of Latin squares. The method for determining the variances of the 3-year means was that of Fisher,[6] recommended by Cochran,[7] and shown in table 1.

In table 1 which represents the actual yields of prime cut corn on the plot basis (0.0102 acre) the sums of the squares for years were obtained by squaring the 3 annual plot totals, each representing the sum of the yields of 144 plots, and subtracting the correction factor consisting of the 3-year grand total divided by 3×144. Two degrees of freedom for years are allowed.

TABLE 1.—*Computation of the pooled variances of replicated Latin squares and subdivision of interaction basis of weight of prime cut corn on plot basis*

INTERACTION

Variations due to—	Degrees of freedom	Sums of squares	Mean square	Standard error
Years	2	5,173.70	2,586.85	
Type of planting	11	2,693.32	244.85	
Interaction	22	306.46	[1] 13.93	[1] 3.73
Total	35	8,173.48		
Tiers	33	1,391.70	42.17	
Columns	33	1,698.15	51.46	
Pooled error mean square	330	1,477.37	4.48	2.12
Total	396	4,567.22		

SUBDIVISION OF INTERACTION

Variations due to—	Degrees of freedom	Sums of squares	Mean square	Standard error
Anomalous behavior of 44 plantings in 1938	1	93.72	93.72	
Remainder	21	212.74	10.13	3.18
Total	22	306.46	[1] 13.93	

[1] Interaction mean square and standard error.

The sums of squares for types of planting were obtained by adding the squares of the 12 3-year type-of-planting totals and subtracting the correction factor mentioned above. The sums of squares for tiers and columns were calculated by adding the respective sums of squares for each year.

The interaction mean square (years × type of planting) has been used instead of the pooled error mean square, following Cochran's suggestions. The former measures only the significance of those differences which have been consistent from year to year. Not only are plot errors measured, but also inconsistencies in behavior of the type of planting from year to year. Cochran has further suggested that the pooled error might be combined with the interaction mean square in instances where the latter is no higher than the former. However, the interaction mean square is higher in all cases except that of husked culls, recovery of prime husked ears, and recovery of prime cut corn. In all three instances the *F* values are not significant and, therefore, the planting rates do not differ significantly.

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[4] See footnote 3, p. 212.

[5] SNEDECOR, G. W. STATISTICAL METHODS APPLIED TO EXPERIMENTS IN AGRICULTURE AND BIOLOGY. Rev. ed., 388 pp., illus. Ames, Iowa. 1938.

[6] FISHER, R. A. THE DESIGN OF EXPERIMENTS. 252 pp., illus. Edinburgh and London. 1935. (See pp. 211-215.

[7] Correspondence with W. G. Cochran, Statistical Laboratory, Iowa State College of Agriculture and Mechanical Arts.

The differences required for significance at the 0.05 and 0.01 levels were determined from Fisher's table of *t* which was entered at 110 degrees of freedom for the annual means and at 22 degrees of freedom (types of planting × years) for the 3-year means.

EXPERIMENTAL RESULTS

GENERAL ANALYSIS OF DATA

The yields have been expressed in two ways, namely, the means and the percentages of the general mean. The former method shows wider differences in the high yielding year 1938 than in 1937 or 1939, the range from highest to lowest for weight of prime cut corn alone in table 4 being 642, 1,106, and 745 pounds per acre for 1937, 1938, and 1939, respectively. The annual changes in the range of differences undoubtedly contribute to the types of planting × years interaction. For the same years in table 4 the percentage differences of prime cut corn are much more stable, being respectively 39.15, 46.85, and 45.38.

The experimental treatments fall into three main groups which may be studied either separately, or collectively, as follows: (1) Alternate hills completely missing; (2) increasing planting rates in uniform stands; (3) irregular stands, all hills planted. Judged, however, on the basis of the yields of the most important yield components (weights of sorted unhusked ears, prime husked ears, and prime cut corn) in tables 2, 3, and 4, the first group is the only one distinctly separate, inasmuch as the three treatments, 0–4, 0–3, and 0–2 are consistently the lowest producers. Groups 2 and 3 fail to show any distinctly

TABLE 2.—*Actual and relative acre weights of sorted unhusked ears of Country Gentleman sweet corn under specified variable and uniform planting rates and distributions for the 3-year period, 1937–39*

Type of planting	1937			1938			1939			3-year average		
	Annual mean yield	Percent of general mean	Rank	Annual mean yield	Percent of general mean	Rank	Annual mean yield	Percent of general mean	Rank	Yield	Percent of general mean	Rank
	Tons			*Tons*			*Tons*			*Tons*		
0–2	2.148	77.43	12	2.597	70.28	12	1.973	70.97	12	2.239	72.62	12
0–3	2.268	81.76	11	3.021	81.76	11	2.204	79.28	11	2.498	81.02	11
0–4	2.329	83.96	10	3.199	86.58	10	2.431	87.44	10	2.653	86.05	10
1–2	2.816	101.51	8	3.528	95.48	9	2.548	91.65	9	2.964	96.14	9
1–3	2.976	107.28	4	3.716	100.57	8	2.723	97.95	8	3.138	101.78	8
1–4	2.902	104.61	6	3.871	104.76	6	2.834	101.94	7	3.202	103.86	6
2–2	2.915	105.08	5	3.885	105.14	5	3.060	110.07	4	3.286	106.58	5
2–3	2.790	100.58	9	3.723	100.76	7	3.010	108.27	5	3.174	102.95	7
2–4	3.079	110.99	2	4.247	114.94	3	3.143	113.05	3	3.490	113.20	2
3–3	3.068	110.60	3	3.920	106.09	4	3.274	117.77	1	3.420	110.93	3
3–4	3.110	112.11	1	4.313	116.72	2	3.155	113.49	2	3.526	114.37	1
4–4	2,884	103.97	7	4.318	116.86	1	3.009	108.24	6	3.404	110.41	4
General mean	2.774	------	---	3.695	------	---	2.780	------	---	3.083	------	---
F value	**16.77	------	---	**31.43	------	---	**23.21	------	---	**21.69	------	---
Standard error	.0818	2.95	---	.0951	2.57	---	.0853	3.07	---	.0892	2.89	---
Difference required for significance:												
At the 1 percent level	.3034	10.94	---	.3527	9.53	---	.3164	11.39	---	.3556	11.53	---
At the 5-percent level	.2292	8.27	--	.2665	7.20	---	.2391	8.60	---	.2616	8.49	---

**=highly significant.

separate tendencies. In other words, evenness of stand is not necessarily the principal criterion of production.

TABLE 3.—*Actual and relative acre weights of prime husked ears of Country Gentleman sweet corn under specified variable and uniform planting rates and distributions for the 3-year period, 1937–39*

Type of planting	1937			1938			1939			3-year average		
	Annual mean yield	Percent of general mean	Rank	Annual mean yield	Percent of general mean	Rank	Annual mean yeld	Percent of general mean	Rank	Yield	Percent of general mean	Rank
	Tons			*Tons*			*Tons*			*Tons*		
0-2	1.445	75.34	12	1.908	70.12	12	1.291	72.37	12	1.548	72.30	12
0-3	1.541	80.34	11	2.183	80.23	11	1.411	79.10	11	1.712	79.96	11
0-4	1.569	81.80	10	2.341	86.03	10	1.563	87.61	10	1.824	85.19	10
1-2	1.949	101.62	8	2.589	95.15	9	1.617	90.64	9	2.052	95.84	9
1-3	2.078	108.34	4	2.766	101.65	7	1.756	98.43	8	2.200	102.76	8
1-4	2.014	105.01	5	2.820	103.64	6	1.817	101.85	7	2.217	103.55	6
2-2	2.000	104.28	6	2.865	105.29	5	1.941	108.80	4	2.269	105.98	5
2-3	1.934	100.83	9	2.748	100.99	8	1.926	107.96	5	2.203	102.90	7
2-4	2.147	111.94	3	3.129	114.99	3	2.026	113.56	3	2.434	113.68	2
3-3	2.159	112.56	2	2.902	106.65	4	2.128	119.28	1	2.396	111.91	3
3-4	2.192	114.29	1	3.200	117.60	2	2.031	113.85	2	2.474	115.55	1
4-4	1.987	103.60	7	3.202	117.68	1	1.901	106.56	6	2.363	110.37	4
General mean	1.918	--	--	2.721	--	--	1.784	--	--	2.141	--	--
F value	**18.08	--	--	**33.63	--	--	**20.42	--	--	**18.04	--	--
Standard error	.0603	3.14	--	.0701	2.57	--	.0578	3.25	--	.0706	3.30	--
Difference required for significance:												
At the 1-percent level	.2237	11.65	--	.2600	9.53	--	.2144	12.05	--	.2814	13.14	--
At the 5-percent level	.1689	8.80	--	.1965	7.20	--	.1620	9.11	--	.2070	9.67	--

** = highly significant.

TABLE 4.—*Actual and relative acre weights of prime cut kernels of Country Gentleman sweet corn under specified variable and uniform planting rates and distributions for the 3-year period, 1937–39*

Type of planting	1937			1938			1939			3-year average		
	Annual mean yield	Percent of general mean	Rank	Annual mean mean yield	Percent of general mean	Rank	Annual mean yield	Percent of general mean	Rank	Yield	Percent of general mean	Rank
	Pounds			*Pounds*			*Pounds*			*Pounds*		
0-2	1,244	75.85	12	1,682	71.24	12	1,215	73.99	12	1,380	73.37	12
0-3	1,338	81.59	11	1,914	81.07	11	1,305	79.48	11	1,519	80.75	11
0-4	1,344	81.95	10	2,048	86.74	10	1,440	87.70	10	1,610	85.59	10
1-2	1,672	101.95	7	2,239	94.83	9	1,507	91.78	9	1,806	96.01	9
1-3	1,755	107.01	4	2,379	100.76	7	1,625	98.96	8	1,920	102.07	8
1-4	1,705	103.96	6	2,438	103.26	6	1,669	101.64	7	1,937	102.98	7
2-2	1,709	104.21	5	2,446	103.60	5	1,793	109.20	4	1,983	105.42	5
2-3	1,668	101.71	8	2,363	100.08	8	1,788	108.89	5	1,939	103.08	6
2-4	1,853	112.99	2	2,725	115.42	3	1,855	112.97	2	2,144	113.98	2
3-3	1,846	112.56	3	2,537	107.45	4	1,960	119.37	1	2,115	112.44	3
3-4	1,886	115.00	1	2,768	117.24	2	1,850	112.67	3	2,168	115.26	1
4-4	1,662	101.34	9	2,788	118.09	1	1,697	103.35	6	2,049	108.93	4
General mean	1,640	--	--	2,361	--	--	1,642	--	--	1,881	--	--
F value	**14.27	--	--	**28.26	--	--	**16.16	--	--	**17.58	--	--
Standard error	56.8	3.46	--	64.7	2.74	--	57.8	3.52	--	60.8	3.23	--
Difference required for significance:												
At the 1-percent level	210.7	12.83	--	240.0	10.16	--	214.4	13.06	--	242.4	12.89	--
At the 5-percent level	159.2	9.70	--	181.3	7.68	--	162.0	9.86	--	178.3	9.48	--

=highly significant.

In most instances the yields rank in much the same relative order from year to year, but in the case of the weights of sorted unhusked ears, prime husked ears, and prime cut corn, discrepancies in the yields of certain treatments must be considered. The weights of sorted unhusked ears, prime husked ears, and prime cut corn in tables 2, 3, and 4, respectively, are unusually consistent with reference to each other within single years as indicated by the relative rankings. Comparisons of rankings from year to year show that the various rates of planting are consistent with the three exceptions discussed below.

The 1–3 rate of planting ranked uniformly fourth in 1937, varied from seventh to eighth in 1938, and was uniformly eighth in 1939.

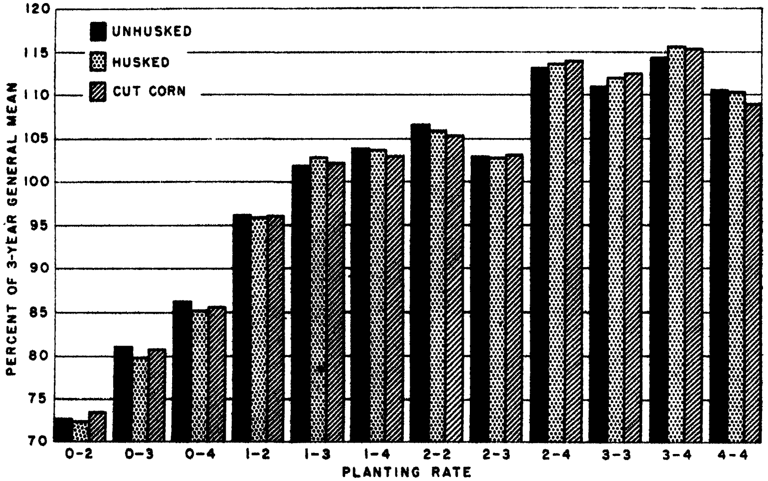

FIGURE 1.—Relation between type of stand and 3-year mean yields of sweet corn expressed as percentages of the general mean in a Latin square, plotted from data in tables 2, 3, and 4. Significant differences at the 5-percent level are 8.49 percent for weights of sorted unhusked ears (*A*), 9.67 percent for prime husked ears (*B*), and 9.48 percent for prime cut corn (*C*).

The data in tables 2, 3, and 4 show, however, that in 1937 the differences between fourth and eighth rankings are not significant. Therefore, the 1937 discrepancy is of minor importance.

The 2–3 planting rate in tables 2, 3, and 4 ranks uniformly fifth for 1939, which is considerably higher than the eighth to ninth ranking in 1937 and the seventh to eighth in 1938. The differences between fifth and eighth ranking in 1939 are significant, but not in 1937 and 1938. However, figure 1 shows that the 2–3 rate has an unduly low 3-year mean yield, indicating a depression of yield in 1937 and 1938 rather than an unusual increase in 1939. Inasmuch as the differences between the fifth and eighth rankings in 1937 and 1938 are not significant, the discrepancy cannot be considered a true anomaly.

The 4–4 rate of planting, however, presents a true anomaly, inasmuch as it varied from seventh to ninth rank in 1937, was uniformly sixth in 1939, but reversed its trend to reach first in 1938. Undoubtedly this anomalous performance has a biological explanation. The general means in tables 2, 3, and 4 show that 1938 was an exceptionally favorable year as compared with 1937 and 1939. Consequently, under optimum conditions a heavy rate of planting such as 4–4 might give exceptionally high yields, reverting to a much lower level of production under less favorable conditions. In other words, the 4–4 planting rate may be considered a borderline treatment, requiring exceptional conditions to produce the maximum weight of ears. That the 4–4 rate will produce a large number of ears even under average conditions has been demonstrated by the writer[8] and is also shown by the data from this experiment in table 6. The number of unhusked and husked prime ears per acre ranked first in 1938 and ranged from second to third in 1937 and 1938, but without any significant differences between first and third ranks in 1937 and 1938.

In other words, the ears from planting rate 4–4 merely developed to larger size in 1938, and the weights per unhusked ear averaged 94.00 percent of the general mean in 1938 as compared with 84.62 and 91.84 percent in 1937 and 1939, respectively. Similarly, the weights per prime husked ear averaged 94.61 percent in 1938, 85.79 percent in 1937, and 91.52 percent in 1939. In all 3 years treatment 4–4 had uniformly the lightest weight ears.

The reversal of planting rate 4–4 from low ranking in 1937 and 1939 to first in 1938 so far as weights of sorted unhusked ears, prime husked ears, and prime cut corn are concerned, contributes to the treatments × years interaction. The interaction may be subdivided, following Snedecor[9] in Section 15.9, as shown in table 1. The method consisted of multiplying each of the 3 yearly totals of 12 replications of treatment 4–4 by 12 and subtracting the respective yearly total yields of the 144 plots in the experiment. The 3 differences thus obtained were calculated according to the following:

$$\frac{[(2\times 1938 \text{ difference}) - (1937+1939 \text{ differences})]^2}{22\times 432=9504} =$$

mean square for treatment 4–4 in 1938.

This product was subtracted from the interaction mean square of years × type of planting.

The ratios between the mean squares for the single degree of freedom and the 21 degrees of freedom as shown in table 1 give the following *F* values:

Weight of sorted unhusked ears	8. 37
Weight of prime husked ears	10. 49
Weight of prime cut corn	9. 25

All of these are highly significant.

EFFECT OF STAND IRREGULARITIES ON YIELDS OF PRINCIPAL EAR COMPONENTS

WEIGHTS OF SORTED UNHUSKED EARS, PRIME HUSKED EARS, AND PRIME CUT CORN

The percentages of the general means from tables 2, 3, and 4 have been plotted in figure 1. The treatment sequence used in plotting figure 1 is the same as that which appears in the tables. According to this sequence the yields increase with a marked degree of regularity through the first seven planting rates, that is, as far as rate 2–2. Beyond this point there is considerable irregularity. It should be noted that all three components of yield are in remarkably close agreement.

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[8] See footnote 2, p. 211.
[9] See footnote 5, p. 213.

More complete comparisons were possible by plotting the data from tables 2, 3, and 4 in five groups holding one of the alternate pairs of hills constant and permitting the second to vary as follows:

		2–0	3–0	4–0
		2–1	3–1	4–1
0–2	1–2	2–2	3–2	4–2
0–3	1–3	2–3	3–3	4–3
0–4	1–4	2–4	3–4	4–4

These groupings involved 21 rates of planting. Since there were only 12 rates in the experiments, 9 were used twice. Three of the 5 groups included uniform rates of planting. If uniformity of planting had a strong influence on yields, the uniform rates in each of the three groups unhusked ears, husked ears, cut corn—should have given unusually high yields, but this did not prove to be the case.

The data for the three yield components show that thickness of stand rather than uniform planting rate is the dominant factor affecting yield. In this respect the results are very similar to those of Kiesselbach and Weihing,[10] who compared systematically variable stands with uniform stands of field corn over a 14-year period and found that the difference averaged only 0.1 bushel per acre.

The data are all very consistent in showing the dominant effect of stand. The 2–3 planting rate is the only one disturbing the regularity. The reasons for this disturbance have already been discussed.

The data in tables 2, 3, and 4 show that the five highest ranking rates on the basis of 3-year mean yields are 3–4, 2–4, 3–3, 4–4, and 2–2 in the order given. In the case of weights of sorted unhusked ears and prime husked ears, all five of these rates have yield differences less than the 5-percent level of significance, but on the basis of prime cut corn weights in table 4, the 2–2 rate has a barely significant lower yield than 3–4, the highest in the group. However, owing to the anomalous behavior of treatment 4–4 in 1938, the interaction may be subdivided and 4–4 removed from consideration in comparing other rates. Using 21 degrees of freedom, the differences required for significance at the 5-percent level between the 3-year means drop to 7.47, 8.20, and 8.24 percent, respectively, in tables 2, 3, and 4. On this basis the 2–2 rate is significantly lower than the top ranking 3–4 rate for all three yield components, but at the same time is not significantly higher than the next heavier rate 2–3.

This is the only point in the experiment excluding the missing hill rates where there is the slightest indication that a uniform rate may be superior to a corresponding irregular rate, that is, one having the same number of hills per plot. However, further analysis in table 5 fails to show that the 2–2 rate is superior because of its uniformity.

The present experiment is complicated by the fact that two variables must be considered. The first of these, uniformity of stand, has already been discussed. The second, number of plants per acre, has not been considered separately. For each pair of hills the planting rate varies from two to eight by single steps. In other words there are seven different thicknesses of planting in the experiment. The weights of the principal ear components are arranged in table 5 on the basis of number of plants in each pair of hills. Four of the seven groups contain more than one type of plant distribution.

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[10] KIESSELBACH, T. A., and WEIHING, R. M. EFFECT OF STAND IRREGULARITIES UPON THE ACRE YIELD AND PLANT VARIABILITY OF CORN. Jour. Agr. Res. 47: 399–416, illus. 1933.

The 0–3 and 0–4 rates are consistent in being significantly lower than any of the other plantings within their respective groups. Apparently where an irregular stand consists of a missing hill it falls into an entirely different category than an irregular stand having all hills planted, even if there is only one plant in the irregular hill. The remaining rates within each group fail to differ significantly in weight of any of the three principal yield components. This indicates that stand irregularity fails to produce significant differences in weights of sorted unhusked ears, prime husked ears, and prime cut corn when the total number of plants per unit of area is held constant. In other words when thickness of stand is held constant, irregularity has no significant influence on yields, but the missing hill type of irregularity causes significant decreases.

TABLE 5.—*Effects of irregularities within classified stand groups on the yields of sweet corn* [1]

Number of plants per 2 hills	Rate	Yield				
		Weight of unhusked ears [2]	Weight of prime husked ears [2]	Weight of prime cut corn [2]	Number of sorted unhusked ears [3]	Number of prime husked ears [3]
		Percent	*Percent*	*Percent*	*Percent*	*Percent*
2	0–2	72.62	72.30	73.37	69.34	69.16
3	0–3	81.02	79.96	80.75	78.43	77.21
	1–2	96.14	95.84	96.01	91.98	92.00
4	0–4	86.05	85.19	85.59	85.98	85.78
	1–3	101.78	102.76	102.07	98.29	99.15
	2–2	106.58	105.98	105.42	103.13	102.44
5	1–4	103.86	103.55	102.98	105.73	105.72
	2–3	102.95	102.90	103.08	102.23	102.29
6	2–4	113.20	113.68	113.98	115.07	115.17
	3–3	110.93	111.91	112.44	111.06	112.27
7	3–4	114.37	115.55	115.26	117.80	118.66
8	4–4	110.41	110.37	108.93	120.96	120.10

[1] Expressed in percentages of the general 3-year mean.

[2] Significant differences at the 5-percent level are 8.49 for weight of unhusked ears, 9.67 for weight of prime husked ears, and 9.48 for weight of prime cut corn, but in the comparisons which do not involve the 4–4 rate the significant differences are 7.47, 8.20, and 8.24 respectively.

[3] Significant differences at the 5-percent level are 5.66 for number of sorted unhusked ears, and 6.34 for number of prime husked ears.

NUMBER OF SORTED UNHUSKED AND PRIME HUSKED EARS

In experimental work dealing with sweet corn, the yields in terms of number of ears per acre must also be considered since truck growers sell by count and not by total weight as cannery growers do. The yields on the basis of numbers of ears are summarized in table 6.

The general trends of yields expressed as number of sorted unhusked and prime husked ears are very similar to those shown when these yields were expressed by weight except for the fact that the 4–4 planting rate is the highest and its behavior was consistent during the 3 years of the experiment. This agrees with the previous work of the writer [11] in which it was shown that the four-per-hill rate is the one to use for Country Gentleman where maximum number of ears

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[11] See footnote 2, p. 211.

TABLE 6.—*Actual and relative 3-year mean acre yields of Country Gentleman sweet corn in terms of number of sorted unhusked and prime husked ears, weight per sorted unhusked and prime husked ear, and weight of unhusked culls planted under specified variable and uniform rates and distributions*

Type of planting	Ears—						Weight—						Unhusked culls		
	Sorted unhusked			Prime husked			Sorted unhusked			Prime husked					
	Per acre	Percent of general mean	Rank	Per acre	Percent of general mean	Rank	Per ear	Percent of general mean	Rank	Per ear	Percent of general mean	Rank	Weight per acre	Percent of general mean	Rank
	Number			*Number*			*Pound*			*Pound*			*Tons*		
0-2	6,503	69.34	12	6,240	69.16	12	0.6861	104.52	1	0.4919	104.42	1	0.207	46.73	12
0-3	7,355	78.43	11	6.966	77.21	11	.6764	103.05	4	.4861	103.18	3	.283	63.88	11
0-4	8,063	85.98	10	7.739	85.78	10	.6544	99.70	7(8)	.4672	99.17	8	.366	82.62	10
1-2	8,626	91.98	9	8,300	92.00	9	.6856	104.45	2	.4889	103.78	2	.373	84.20	9
1-3	9,218	98.29	8	8,945	99.15	8	.6747	102.79	5	.4853	103.01	5	.437	98.65	8(7)
1-4	9,915	105.73	5	9,538	105.72	5	.6425	97.88	9	.4589	97.41	10	.437	98.65	7(8)
2-2	9,672	103.13	6	9,242	102.44	6	.6769	103.12	3	.4858	103.12	4	.443	100.00	6
2-3	9.587	102.23	7	9.229	102.29	7	.6575	100.17	6	.4722	100.23	6	.509	114.90	4
2-4	10,791	115.07	3	10,391	115.17	3	.6417	97.76	10	.4619	98.05	9	.505	114.00	5
3-3	10,415	111.06	4	10,129	112.27	4	.6544	99.70	8(7)	.4708	99.94	7	.539	121.67	3
3-4	11,047	117.80	2	10,706	118.66	2	.6328	96.40	11	.4558	96.75	11	.590	133.18	2
4-4	11,344	120.96	1	10.835	120.10	1	.5936	90.43	12	.4283	90.91	12	.625	141.08	1
General mean	9,378			9.022			.6564			.4711			.443		
F value	**68.51			**56.93			**.0891			**.0961			**8.92		
Standard error	181	1.93		195	2.16		.0089	1.36		.0059	1.25		.041	9.26	
Difference required for significance:															
At the 1-percent level	721	7.69		777	8.61		.03548	5.41		.02352	4.99		.1634	36.88	
At the 5-percent level	531	5.66		572	6.34		.02610	3.98		.01730	3.67		.1202	27.13	

**Highly significant.

per acre is the primary consideration. Accordingly, larger yield differences may be expected as a result of the greater effect of total stand. This is what actually occurred. In the case of yields in terms of number of sorted unhusked ears, the 4–4 and 3–4 rates are significantly the highest in the entire experiment and do not differ statistically. On the basis of yields of number of prime husked ears, there are three statistically identical rates which are significantly higher than all the remaining rates, namely, 4–4, 3–4, and 2–4. Here, again, uniformity of stand is not the criterion of yield.

Further analysis of the data on number of sorted unhusked and prime husked ears in table 5 shows that the missing-hill type of stand irregularity falls into a category different from that of the irregular stands in which all the hills are planted, inasmuch as the yields are significantly lower than the rest of the treatments in their respective groups. In fact the latter show no significant differences.

WEIGHT PER SORTED UNHUSKED AND PRIME HUSKED EAR

The summarized 3-year mean weights per ear shown in table 6 are obviously influenced primarily by number of plants per hill, decreasing inversely with respect to number of plants per hill. None of the decreases are statistically significant, however, except where the planting distribution included four plants per hill.

The data may be grouped and analyzed in the same manner as table 5, but not shown here. Significant differences failed to appear, with one exception, when the data were analyzed on such a basis, indicating that when the number of plants was held constant in pairs of hills, their distribution had no significant effect on weight per ear, although there was a distinct tendency toward a decrease where the group member included a hill with four plants. In one of the groups, namely, 0–4, 1–3, and 2–2, the 0–4 member produced significantly smaller prime husked ears than the highest member, 2–2.

RECOVERY OF PRIME HUSKED EARS AND PRIME CUT CORN

To the canner any changes in the percentages of prime husked ears and prime cut corn recovered from unhusked ears are of obvious importance. The data for these components have been computed, but as the F values fall considerably below significance, it may be assumed there is no difference between the rates of planting. These conclusions are in agreement with the results of the writer,[12] who found that the recovery of prime husked ears in the Country Gentleman variety was not significantly changed by increased rates per hill except where the number of plants per hill was large and the planting distances close. In such instances the recovery of prime husked ears decreased. However, both the rates per hill and the distances between rows were beyond the limits of the present experiments.

WEIGHT OF UNHUSKED AND HUSKED CULLS

The yields of culls are of no particular importance except for the fact that their presence is highly objectionable to the canner because of the added cost of handling and of waste disposal. The yields of unhusked culls summarized in table 6 proved to be, with the exception of the 2–2 and 3–3 rates, remarkably uniform from year to year on the basis of the relative rankings. In contrast, the annual yields of husked culls were, with few exceptions, exceedingly divergent.

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[12] See footnote 2, p. 211.

The weights of unhusked culls increase at a rapid rate in relation to the number of plants per hill, but these increases are independent of regularity of stand. Comparisons made in the same manner as in table 5, but not shown, indicate that there are no statistical differences within the four groups containing more than one rate of planting. As a matter of fact, in two of these four groups (0–4, 1–3, and 2–2; 2–4 and 3–3), which are the only ones containing uniform planting rates, the uniform rates have greater weights of unhusked culls than the irregular rates both with and without missing hills.

The weights of husked culls have *F* values considerably below significance. It is assumed, therefore, that neither thickness nor irregularity of stand shows significant differences. The fact that thickness of stand fails to increase the weights of husked culls within the limits of the present experiment is in agreement with the work of the writer,[13] who obtained significant increases only in plantings heavier than those tested here.

COMPARISON BETWEEN UNIFORM AND MISSING HILL STANDS

Comparisons on the basis of the general mean have been made between uniform and irregular rates of planting, the latter including stands with alternate missing hills. The effect of alternate missing hills on yields may be considered separately on the basis of comparisons with the corresponding uniform planting rate and ignoring the general mean. Three pairs of treatments may be compared, namely, 2–2 and 0–2, 3–3 and 0–3, 4–4 and 0–4. These comparisons are summarized in table 7. The reductions in the total yields due to missing hills are progressive, being lowest where the stand is four per hill and highest where it is two per hill. Weight per ear is not only greater in missing hill stands, but also shows a progressive tendency in relation to rate opposite to that of total yields.

TABLE 7.—*Changes in 3-year mean yields due to alternate missing hills in 3 stands of sweet corn* [1]

Yield components	Change due to missing hills in indicated stand		
	2	3	4
	Percent	*Percent*	*Percent*
Weights:[2]			
Sorted unhusked ears	**31.86	**26.96	**22.06
Prime husked ears	**31.78	**28.55	**22.81
Prime cut corn	**30.41	**28.18	**21.42
Unhusked culls	**53.27	**47.50	**41.44
Number:[2]			
Sorted unhusked	**32.76	**29.38	**28.92
Prime husked	**32.48	**32.21	**28.57
Weight per ear:[3]			
Sorted unhusked	1.36	*3.36	**10.24
Prime husked	1.26	**3.25	**9.08

[1] Yields are expressed as percentages calculated from the formula $\frac{a-b}{a} \times 100$ where *a* and *b* are the 3-year mean yields of the respective complete and alternate missing hill stands.

[2] All figures given are decreases.

[3] All figures given are increases.

*Significant at 5-percent level.

**Significant at 1-percent level.

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[13] See footnote 2, p. 211.

MATURITY IN RELATION TO STAND

The writer has previously shown [14] that increasing the number of plants per hill tends to delay maturity. Such a tendency was also noted in the present experiment. Data for 2 of the 3 years, 1937 and 1938, are shown in table 8. These data were computed from silk counts taken every other day during the early maturation period. Methods of making such counts and their interpretation in terms of maturities are discussed by Huelsen and Michaels.[15] Owing to the 2-day intervals between counts it proved to be impractical to interpret maturities in terms of time units. The data in table 8 have, therefore, been expressed as percentages above or below the theoretical number of silks (75 percent of the total) at midsilking for each planting rate on the day the entire experiment actually reached midsilking. The 2–4 rate may be used as an example. The silks of the 2 center rows (total of 20 hills) were counted, a procedure uniformly followed. The 20 hills of the 2–4 rate contained 60 plants, or a total of 720 plants in 12 replications. It was assumed that 75 percent of 720 plants, namely, 540, would be the number of silks showing at the midsilking point. The validity of this procedure is discussed by Huelsen and Michaels. Since 540 silks was the theoretical midsilking point and 556 were actually counted on August 15, 1938, the midsilking point of the entire experiment, the percentage was calculated as follows:

$$\frac{556}{540} \times 100 = 102.96 \text{ percent.}$$

The 2–4 rate had 2.96 percent more than 75 percent of the theoretical number of silks showing on August 15 and, therefore, was very slightly earlier than the entire experiment.

TABLE 8.—*Relation between maturity of sweet corn and type of stand* [1]

1937

Number of plants in alternate (varying) hill	Number of plants per hill, stand constant				
	0	1	2	3	4
			65.56	40.74	−1.74
			51.11	30.28	−.67
	65.56	51.11	45.28	3.78	2.22
	40.74	30.28	3.78	11.48	−5.24
	−1.74	−.67	2.22	−5.24	−25.69
0			48.89	28.52	8.61
1			25.92	20.28	−6.22
2	48.89	25.92	42.22	−4.67	2.96
3	28.52	20.28	−4.67	−.93	−6.20
4	3.61	−6.22	2.96	−6.20	−25.42

[1] Expressed as percentage increase (earlier maturity) or decrease (later maturity) in relation to theoretical number of silks at midsilking.

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[14] See footnote 2, p. 211.

[15] HUELSEN, W. A., and MICHAELS, W. H. THE YIELD COMPLEX OF SWEET CORN. Ill. Agr. Expt. Sta. Bul. 432: pp. 505–608, illus. 1937.

The data in table 8 show a close relationship between maturity and stand, delayed maturity being associated with increased planting rates. There is no conclusive evidence that uniform planting rates have any effect on maturity independent of total stand.

SUMMARY

The effect of irregularities in the field stands of hybrid Country Gentleman sweet corn has been studied over a 3-year period. Twelve combinations of rate and distribution of planting were included, 3 of them uniform, 3 with missing hills, and 6 with a constant rate of 1, 2, 3, or 4 plants in one hill and the next one varying. The 12 stand variations were laid out in the field on the Latin-square basis with 12 replications. The experiment was moved to a new site each year.

Total number of plants per acre rather than irregularity of distribution within reasonable limits was the factor which determined yields. The yield components thus affected were number and weight of sorted unhusked ears, number and weight of prime husked ears, and weight of prime cut kernels.

Weight per unhusked and prime husked ear was influenced primarily by thickness of planting and not by irregularities in stand. Increasing the number of plants per hill reduced the weight per ear.

Recovery of prime husked ears and of prime cut corn proved to be highly variable from year to year and showed no significant trends within the limits of this experiment either with respect to total stand, or irregularities of stand.

Weights of unhusked culls increased rapidly in relation to total stand, but were not affected by irregularities of distribution. Weights of husked culls failed to show any definite trends either with respect to total or irregular stands.

A comparison of yields from uniform stands containing two, three, and four plants per hill with those from equivalent half stands in which alternate hills were missing, showed that significant reductions occurred in all components of yield except weight per ear where significant increases were noted in most cases. Both the decreases and increases also showed distinct trends. Total weights of ears, cut corn, and unhusked culls along with total number of ears all showed the greatest decreases in missing hill stands containing 2 per hill and the smallest for four per hill. This trend was reversed in the case of the weight per ear increases.

Increasing the total stand tended to delay maturity as determined by silk counts. There was no consistent relationship between maturity and irregularities in stand.

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TYPE OF SEED FORMATION AS INDICATED BY THE NATURE AND EXTENT OF VARIATION IN KENTUCKY BLUEGRASS, AND ITS PRACTICAL IMPLICATIONS[1]

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INTRODUCTION

Investigations on both European and American biotypes of Kentucky bluegrass (*Poa pratensis* L.) have established the fact that seed formation is predominantly apomictic; that is, the embryo develops solely from maternal tissues without previous fertilization of the egg nucleus by a sperm from the pollen. The offspring may thus resemble the mother plant in morphological and physiological characters, since they are essentially clonal increases of it.

Quite apart from its purely scientific interest, the method of seed formation in *Poa pratensis* has important practical implications, as it may supply the answers to such questions as the following: Is improvement in this species to be limited solely to selection of desirable types that already are available in nature? Or do forms exist that allow the breeder to choose from among the various inbreeding and hybridization techniques one particularly adaptable to *Poa*?

The present study was designed to determine the extent and nature of the apomictic and sexual methods of seed production in a representative sample of Kentucky bluegrass obtained from pasture and commercial seed sources in the United States and Canada. It was thought that, by detailed statistical and descriptive analyses of the material and its progenies from both self-pollination and open pollination, some broad generalizations might be reached that could ultimately be applied to practical breeding methods in the improvement of Kentucky bluegrass.

It is hoped that the results presented will indicate more clearly than hitherto (1) the extent of apomixis and sexuality in a representative group of Kentucky bluegrass plants, (2) the nature of the aberrant forms that are produced by sexual phenomena, and (3) the practical application of the results to breeding techniques in *Poa*.

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[1] Received for publication August 22, 1942. The plant material for this study was grown and the field data collected while the writer was a member of the staff of the U. S. Regional Pasture Research Laboratory, State College, Pa., maintained in cooperation with the 12 Northeastern States. The cytological and statistical analyses were made and the manuscript was prepared while the writer was a graduate student in the Department of Agronomy of the University of Maryland. The material presented here is based on a thesis submitted to the Graduate School of the University of Maryland in partial fulfillment of the requirements for the degree of doctor of philosophy.

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TERMINOLOGY OF APOMIXIS

Apomixis is a general term and was defined in 1908, according to Stebbins (*33*),[3] by Winkler, as "the substitution for sexual reproduction of another, asexual reproductive process that does not involve nuclear or cellular fusion." In 1922, according to Fagerlind (*11*), Täckholm proposed the term "agamospermy" for seed production without fertilization. Since, in current literature, apomixis and agamospermy are frequently used synonymously in referring to seed production without fertilization, the writer proposes to use the simpler and more generally understood term "apomixis" when describing asexual seed production in Kentucky bluegrass.

Included under apomixis are the phenomena of haplospory, diplospory, generative and somatic apospory, and adventitious embryony. For complete discussions of the history, descriptions, and terminology of apomictic processes in higher plants the reader is referred to the publications of Gustafsson (*14*, *15*), Stebbins and Jenkins (*34*), Fagerlind (*11*), Gentcheff and Gustafsson (*13*), and Stebbins (*33*).

In discussing apomictic origins, the implication has been made that the offspring will resemble the maternal parent. This is true only if offspring have arisen by diplospory, apospory, or adventitious embryony, since only these processes can result in the establishment of the original chromosome complement of the parent. Haplospory, though an apomictic process, may produce a wide range of morphological types in the offspring. In this paper, however, the expression "apomictically reproduced plants" refers only to those plants that show complete conformity to the mother plant.

In describing the manner of origin and the chromosomal complements of plants of Kentucky bluegrass, the expressions "haploid," "diploid," and "triploid" are employed. This is obviously a loose use of the terms when applied to a collection of biotypes whose chromosome numbers range from $2n=36$ (*22*) to $2n=\pm110$, and whose basic number is $x=7$. However, these terms are convenient and will be employed with the following implications:

Haploid.—A plant having approximately half the number of chromosomes found in the mother plant. Haploids arise by haplosporic parthenogenesis.

Diploid by apomixis.—A plant having the same chromosome number as the parent and conforming to it in morphological and physiological characters. It may arise by either diplosporic parthenogenesis or aposporic parthenogenesis.

Diploid by amphimixis.—A plant whose chromosome number is approximately the same as that found in its parent, and whose morphological, physiological, and cytological features suggest that it arose by the union of a reduced egg and a reduced sperm; that is, by normal sexual reproduction.

Triploid.—A plant having approximately 50 percent more chromosomes than exist in the mother plant. It is generally believed that triploids arise through the fertilization by reduced pollen of an unreduced egg derived by either diplospory or apospory. The possibility may not be wholly eliminated, however, that in *Poa* certain triploids arise through the fertilization of a reduced egg by an unreduced pollen grain (*22*).

REVIEW OF LITERATURE

The first suggestion that seed production in Kentucky bluegrass was predominantly apomictic was made by Müntzing (*19*), who studied eight Swedish biotypes of *Poa pratensis*. Apomictic seed production was also reported in Swedish forms of *P. alpina* L. Müntzing proposed the following criteria for apomixis: (1) An aneuploid chromosome number that is constant for the biotype; (2) morphological constancy within the biotype; and (3) good seed production, even in plants with great chromosome irregularities at meiosis. Apomictic seed formation in *P. pratensis* has been confirmed by Åkerberg (*1, 2, 3, 4*), Engelbert (*9*), Müntzing (*22*), Nilsson (*23, 25*), Rancken (*28*), Tinney (*35*), and Tinney and Aamodt (*36*), and may be inferred from the morphological constancy observed by Armstrong (*6*) and Kemp (*16*). Several other species of *Poa* have been described as containing apomictic biotypes: *P. palustris* L. by Kiellander (*18*); *P. arctica* R. Br., *P. alpigena* (E. Fries) Lindem., and *P. glauca* Vahl, of northern Europe, by Flovik (*12*); *P. arctica*, *P. alpina*, and *P. alpigena*, of Canada, by Engelbert (*9*); and *P. compressa* L., by the writer (*7*).

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[3] Italic numbers in parentheses refer to Literature Cited, p. 262.

Poa pratensis has been shown to be a remarkably diversified species in its range of chromosome numbers. Reports have been made by Åkerberg (*1, 2, 4*), Armstrong (*6*), Avdulow (cited by Brown (*8*), Brown (*8*), Müntzing (*19, 20, 21, 22*), Rancken (*28*), Skovsted (*30*), and Tinney (*35*). The lowest somatic number, $2n=28$, was reported by Avdulow as cited by Brown (*8*); the highest, $2n=110$, by Åkerberg (*4*). Brown (*8*) has compiled all the reports of chromosome numbers in Kentucky bluegrass and finds a very steep mode at $2n=56$, indicating that most plants are octaploid. Observations on meiosis in *P. pratensis* have been reported by Armstrong (*6*), Müntzing (*19, 22*), Rancken (*28*), and Tinney (*35*). In general, the division is highly irregular, although Müntzing (*22*) describes a regular meiosis in a sexual 36-chromosome "haploid."

The origins of the embryo sac and embryo have been reported for *Poa pratensis* by Åkerberg (*4*), Andersen (*5*), Armstrong (*6*), and Tinney (*35*); for *P. compressa* by Andersen (*5*); for *P. palustris* by Kiellander (*18*); for *P. alpina* by Müntzing (*22*); and for *P. arctica* by Engelbert (*10*). Kiellander and Müntzing described diplospory in *P. palustris* and *P. alpina;* Åkerberg and Tinney, somatic apospory in *P. pratensis;* Engelbert, somatic apospory in *P. arctica;* and Åkerberg, Andersen, Armstrong, Müntzing, and Engelbert described or suggested sexual reproduction in the species with which each worked.

Somatic apospory as the basis of apomixis in *Poa pratensis* was first described by Åkerberg (*4*). In an apomictic biotype, the embryo sac arose from a cell of the nucellus, producing an egg with the unreduced number of chromosomes, which then developed parthenogenetically. Tinney (*35*) reported in greater detail the same series of phenomena. In all observed cases the megaspores degenerated and an embryo sac formed from an enlarged cell of the nucellus, the unreduced egg of which developed parthenogenetically to form the embryo. In many instances, the embryo was well formed before flowering, and Tinney suggested that the development of the embryo is not pseudogamous but that, since the endosperm does not develop until later, the stimulus of pollen may be necessary for endosperm formation and subsequent seed formation. Some embryo sacs are slow in development and may not have formed an embryo at flowering. It was suggested that triploids might arise from the fertilization of these slowly developed egg cells. The origin of twin embryo sacs was attributed to the simultaneous development of two nucellar cells. In this interpretation Tinney differs from Andersen (*5*), who described twins derived from two megaspores, and from Engelbert (*10*), who stated that, in *P. arctica*, twins arise by the development of a reduced megaspore and an unreduced nucellar initial. Åkerberg (*4*) reported embryological studies on a sexual biotype of *P. pratensis* and found complete absence of aposporous development. All embryo sacs were derived from functional reduced megaspores.

Self- and cross-fertility in *Poa pratensis* have been analyzed by Nilsson (*23, 24, 25*). He has shown that plants differ widely in their ability to set seed under bag. He concluded that, while the physiological effect of the bag on seed set may be considerable under some conditions, true differences in self-sterility and self-fertility exist among plants of Kentucky bluegrass and that genotypical differences are responsible.

All experimental evidence leaves little doubt that pollination is necessary for seed production in *Poa pratensis*. Åkerberg (*1, 2*) and Nilsson (*25*), working with sterile apomictic biotypes, found that, regardless of the type of *Poa* pollen applied to the stigma, offspring were matroclinous. In rare instances, hybrids were obtained. Results obtained by the writer (*7*), by applying *P. pratensis* pollen on heat-emasculated florets of *P. compressa*, indicate similar pseudogamous development and occasional fertilization. Åkerberg (*4*) and Engelbert (*9*) reported results of hand emasculation on apomictic biotypes in several species of *Poa*. In no case was seed produced unless pollen was applied.

Numerous references have been made to the high frequency of polyembryony in Kentucky bluegrass. It was first mentioned by Nishimura (*26*). Andersen (*5*), Armstrong (*6*), Engelbert (*10*), and Tinney (*35*) have described and interpreted the embryology of twin seedlings in species of *Poa*. Müntzing (*20, 21, 22*) has presented a very complete analysis of the morphology and chromosome complements of twin seedlings of *P. pratensis*. Skovsted (*30*) has made an extensive cytological study of twin seedlings.

Åkerberg (*4*) reported on the occurrence of polyembryony in progenies of *Poa pratensis*. He found it a highly variable feature among his plants, stated the average frequency as about 10 percent, and suggested that the sexual types show a significantly lower rate of polyembryony than do the predominantly apomictic types. Åkerberg reported 12.7 percent aberrancy in plants from twin-seedling sources, as compared with 6.9 percent in plants from single-seedling sources.

Webber (*38*) has prepared a review of the subject of polyembryony in the higher plants.

Morphological variation in progenies of Kentucky bluegrass and the chromosome complements of the aberrant plants have been investigated by Åkerberg (*4*). Selfed progenies showed a variability of 12.1 percent; artificially crossed progenies, 13.4 percent. Fifty-eight of the plants of the offspring were investigated cytologically; 43 were of maternal types and had the same chromosome numbers as the parents, and 6 had chromosome numbers suggesting origin by triploidy. Many of the aberrant F_1 plants gave highly variable F_2 progenies, and the suggestion is made that the aberrant plants were more sexual than the parental type.

Tinney and Aamodt (*36*) have published the results of 102 progeny tests conducted on Kentucky bluegrass material collected from sources in North America and Europe. Collections from pastures were included. The progenies from 48 of the selected plants were uniform and the 2 highest values obtained for morphological variability were 12.06 percent and 21.92 percent. The entire nursery showed an average value for variability of 1.59 percent; 31 progenies from Wisconsin pasture sources gave a value of 1.65 percent. Tinney and Aamodt suggested that the variant plants had arisen by either gametic union or mutation.

Techniques that facilitate the identification of plants with aberrant chromosome numbers have been described. Müntzing (*22*) has presented evidence for the high positive correlation between chromosome number and diameter of pollen grains in *Poa pratensis*. Nissen (*27*) similarly showed a positive correlation between chromosome number and size of stomata in this species. Both of these correlations have been shown by Müntzing (*22*) to exist also in *P. alpina*.

Morphological constancy in the progenies of Kentucky bluegrass is recognized by all investigators in this field as a reliable criterion of apomictic processes. Conversely, aberrant plants that arise are considered as visible proof of sexual reproductive processes. These criteria of reproduction were recognized first by Müntzing (*19*), followed by Åkerberg (*4*) and Tinney and Aamodt (*36*), the latter suggesting the progeny test as a practical means of evaluating types of seed development.

MATERIAL AND METHODS

SOURCES OF MATERIAL

The foundation stock from which selections were made was drawn from widely diversified sources. Detailed information is given in table 1. Seed for the original Kentucky bluegrass nursery of approxi-

TABLE 1.—*Pedigree, foundation stock, and sources of the material from which the Kentucky bluegrass selections were made*

Pedigree	Foundation stock	Source
37-KB 1 to 114	Seed collection	West Virginia pasture.
118	do	New Jersey pasture.
120	do	New Hampshire pasture.
127	do	New York pasture.
181	Sod plug collection	West Virginia pasture.
37-KB 128 to 140	Commercial seed	Kentucky.
142 to 146	do	Missouri.
152	do	Do.
161 to 165	do	Do.
147	do	Kansas.
151 and 156	do	Minnesota.
153	do	South Dakota.
154	do	Iowa.
155	do	Nebraksa.
37-KB(CB)134(470)[1]	do	Canada.
135(119) (156) (231)[1]	do	Do.
138(525)[1]	do	Do.
37-KB(Asp) 19(1)[2]	do	New York.
37-KB 170	Strain	Ottawa 939; Minnesota P-35.
171	do	Ottawa 993; Minnesota P-36.
172	do	Ottawa 994; Minnesota P-37.
175	do	Ontario Agricultural College 1.
176	do	Ontario Agricultural College 2.
177	do	Ontario Agricultural College 3.
37-KB 173	Introduction	F. P. I.[3] 114272.
174	do	F. P. I. 73163.
186	do	F. P. I, 115314 Russia.
187	do	F. P. I. 115405.
37-KB(Psp) 1[4]	do	F. P. I. 95581 Canada.

[1] Kentucky bluegrass plants found in Canada bluegrass progenies.
[2] Kentucky bluegrass plant found in *Agrostis* progeny.
[3] F. P. I. denotes Foreign Plant Introduction.
[4] Introduced as an unnamed species of *Poa*.

mately 10,000 individually spaced plants, established at State College, Pa., in the spring of 1937, came from 4 sources: (1) Seed collections from permanent pastures, 118 pedigrees, from which 28 parental plants were drawn; (2) seed from commercial sources, 38 pedigrees, from which 71 parental plants were drawn; (3) seed of numbered strains and selections, 6 pedigrees, from which 9 parental plants were chosen; and (4) seed of Bureau of Plant Industry introductions, 5 pedigrees, from which 7 parental plants were chosen.

In all, 115 parental plants were selected from among the 10,000 in the nursery. An effort was made to have these plants representative of the range of variation in plant type and response found in the original nursery material.

PROCEDURE

Each of the selected plants of Kentucky bluegrass was bagged in the spring of 1938 to determine the extent of sterility under bag existing in the material and to provide seed for the study of inbred progenies. Open-pollinated seed was also collected.

Seed was germinated in the fall of 1938, 6 weeks after harvest. One hundred seeds were placed on blotting paper in Petri dishes. The rest period of the seed was broken successfully by exposing the moistened seed to a temperature of 8° C. for a period of 10 to 14 days, followed by germination at room temperatures, a procedure found effective by Sprague (*32*). On germination, the single seedlings and twin and triple seedlings were separated and handled separately. The nursery consisted entirely of plants from seeds giving rise to single seedlings.

The single seedlings were transplanted from Petri dishes to paper bands in flats, and allowed to reach sufficient size for transplanting to the field. They were removed to the field in October 1938 during a favorable spell of weather which allowed them to become well established. Progenies were arranged in compact blocks to minimize environmental influence on the plants. With each progeny were planted clonal increases of the parental plant for comparisons of type and vigor. The final data presented in this paper were obtained during the spring and summer of 1940, at which time all plants had reached maturity.

The double and triple seedlings were also transplanted to paper bands and allowed to reach a size best suited for their separation. Each seedling was transplanted to a 3-inch pot. As the plants developed to the point where notes could be taken, those twins and triplets that seemed identical were discarded. Of those that remained, a representative sample was taken for transplanting to the field; 69 pairs of twins and 4 sets of triplets were set out.

EXPERIMENTAL RESULTS

SEED SET UNDER BAG

The 115 plants selected for progeny tests were brought into the greenhouse in the fall of 1938, where 59, or approximately half of them, flowered. As nearly as could be judged, the flowering was normal. Four to eight panicles of each plant were placed in a parchment bag. Seed set was classified on the basis of the percentage of florets that set seed, and 4 identifiable classes were established:

(1) No set, (2) less than 30 percent set, (3) 30 to 60 percent set, and (4) more than 60 percent set.

All 115 plants flowered in the field in 1938, and seed set was tested under parchment bags. Bagging operations were repeated in the field the following year with parchment bags in duplicate. Table 2 presents the data on seed set. The numbers in parentheses refer to the 59 selected plants that flowered previously in the greenhouse. Based on the replicated data, the column headed "Composite data" was set up as the best available appraisal of seed set under bag.

TABLE 2.—*Seed set under bag in 115 selected Kentucky bluegrass plants*

Seed set (percent)	Plants setting seed[1] in—						Composite data	
	Greenhouse		Field, 1938		Field, 1939			
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
None	12	20.3	(9) 16	(15.3) 13.9	(2) 9	(3.4) 7.8	(5) 10	(8.5) 8.7
Less than 30	7	11.9	(12) 23	(20.3) 20.0	(17) 35	(28.8) 30.4	(15) 30	(25.4) 26.1
Subtotal	19	32.2	(21) 39	(35.6) 33.9	(19) 44	(32.3) 38.2	(20) 40	(33.9) 34.8
30-60	4	6.8	(12) 37	(20.3) 32.2	(7) 24	(11.9) 20.9	(7) 30	(11.9) 26.1
More than 60	36	61.0	(26) 39	(44.1) 33.9	(33) 47	(55.9) 40.9	(32) 45	(54.2) 39.1
Subtotal	40	67.8	(38) 76	(64.4) 66.1	(40) 71	(67.8) 61.8	(39) 75	(66.1) 65.2
Total	59	100.0	(59) 115	(100.0) 100.0	(59) 115	(100.0) 100.0	(59) 115	(100.0) 100.0

[1] Numbers in parentheses refer to field data from the 59 plants that flowered under greenhouse conditions.

Remarkably consistent values throughout the series of bagging studies are obtained by combining the classes "no set" and "less than 30 percent set" and the classes "30 to 60 percent set" and "more than 60 percent set." Of the 115 plants tested, only 5 failed to give consistent results throughout the replicated series, so that the 30-percent level of seed set seems to be an expedient biological measure of ability of plants of Kentucky bluegrass to set seed under the bagging conditions encountered in this work.

These bagging tests conducted on 115 plants indicate that 34.8 percent set seed poorly (less than 30 percent) or not at all, and that 65.2 percent set seed well (more than 30 percent). There is no significant difference in ability to set seed under bag, measured at the 30-percent level, between the group of plants that flowered in the greenhouse and the entire group of 115 plants. In this connection, it may be pointed out that greenhouse conditions apparently provided a more satisfactory environment for the determination of sterility than did field conditions, since higher percentages of plants set no seed and set good seed in the greenhouse than elsewhere.

ANALYSIS OF SELF-POLLINATED AND OPEN-POLLINATED PROGENIES

A study was made to determine whether any significant differences exist between progenies from seed produced under bag and progenies from seed produced under conditions of open pollination. Both self-pollinated and open-pollinated progenies were available from 87 parental plants. Data from this analysis of germination, polyembryony, survival, and variability are summarized in table 3.

The values for polyembryony are based on the number of germinated seeds that showed evidence of containing more than one embryo. These were almost entirely twin seedlings, although triple seedlings appeared in some progenies in appreciable numbers. Invariably the appearance of triple seedlings was associated with a high incidence of polyembryony. The correlation between the values for polyembryony in self-pollinated and open-pollinated progenies is very high (fig. 1).

The distributions of the values for polyembryony in 87 self-pollinated and 87 open-pollinated progenies are shown in figure 2, *A*. In order to determine whether significance could be ascribed to any differences existing between paired progenies from each parental plant, chi-square determinations were made and plotted against the theoretical distribution for $N=87$. There is remarkably close agreement, indicating that, although rates of polyembryony in plants of Kentucky bluegrass may show highly significant differences, yet the rate for a particular plant is independent of the type of pollination.

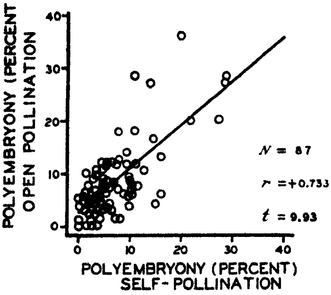

FIGURE 1.—Correlation of polyembryony in progenies from self-pollination with polyembryony in progenies from open pollination.

TABLE 3.—*Statistical data from self-pollinated and open-pollinated progenies of 87 plants*

Item	Correlation[1] index	Mean	Difference[2]	t	Lowest value	Highest value	Standard deviation
Germination:		*Percent*			*Percent*	*Percent*	
Self-pollination	+0.278	82.4±1.6	2.0±2.0	1.00	37.4	100.0	14.5
Open pollination		80.4±1.3			28.0	98.0	11.8
Polyembryony:							
Self-pollination	+.733	6.9±0.7	1.2±1.0	1.23	0	28.7	6.1
Open pollination		8.1± .7			0	36.1	7.0
Survival:							
Self-pollination	+.610	84.9±1.5	1.6±1.9	.83	22.0	100.0	13.6
Open pollination		86.5±1.2			34.0	100.0	11.1
Variability:							
Self-pollination	+.614	10.9±1.4	3.8±2.2	1.71	0	64.6	14.2
Open pollination		14.8±1.5			0	74.6	15.3
Variability after elimination of 3 widely divergent pairs:							
Self-pollination, $N=84$	+.818	10.5±1.4	3.1±2.1	1.49	0	64.6	12.8
Open pollination, $N=84$		13.6±1.5			0	70.6	13.7
Size of progeny (number of plants):		*Number*			*Number*	*Number*	
Self-pollination (4,260)	....	49.0±1.2	.....	..	12	60	10.8
Open pollination (4,437)	....	51.0± .8	.....	..	17	60	7.4

[1] $P=0.01$ at $r=0.27$.
[2] $P=0.05$ at $t=1.96$.

The experimental nursery was planned so that ultimately each progeny would consist of 60 plants in the field. With few exceptions, sufficient seed germinated to supply this number. The studies of the mature plants in the nursery revealed the fact that certain progenies had lost a considerable number of plants and that others had lost none or very few. It was thought advisable to analyze the material to discover, should the loss in plants be the expression of some in-

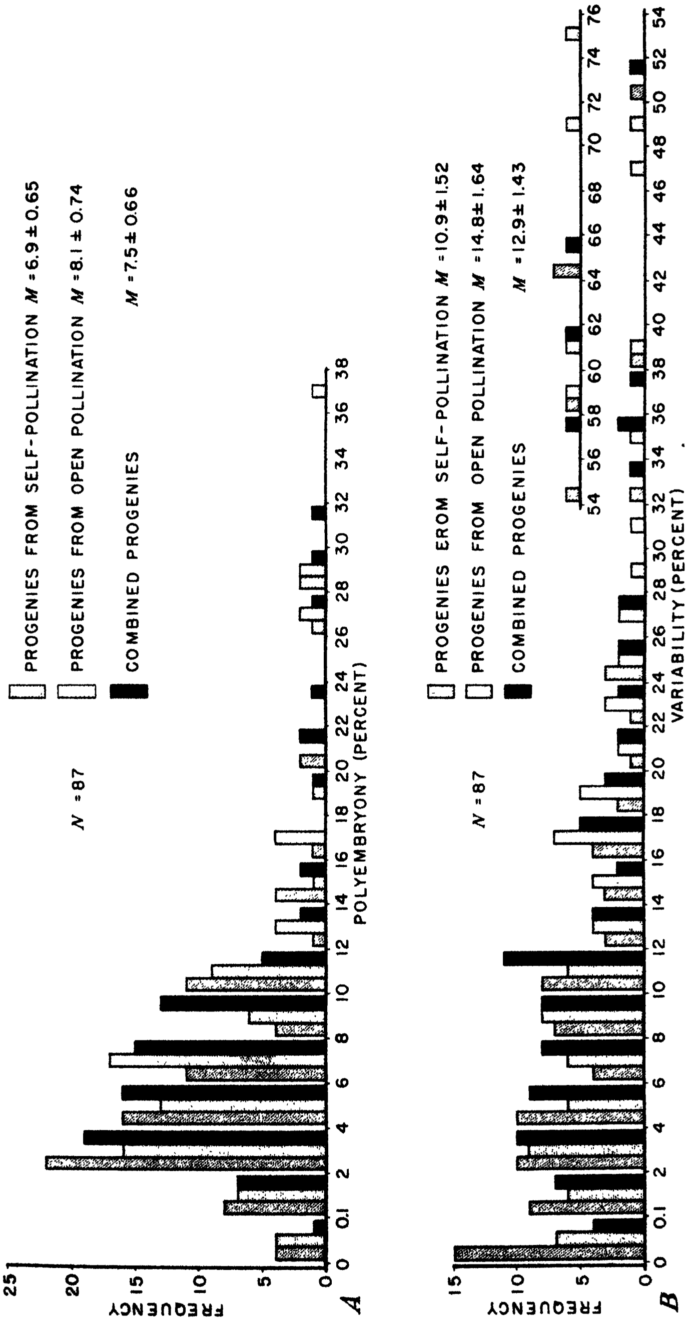

FIGURE 2.—Distribution of values for (A) polyembryony and (B) variability in self-pollinated and open-pollinated progenies of 87 parental plants.

herent character of the parent plant, if any difference existed between self-pollinated and open-pollinated progenies. The results are expressed in percentage survival and include those plants that survived transplanting to flats and transplanting to the field and those that persisted in the field for 2 years.

The high correlation (fig. 3) between percentage survival in self-pollinated progenies and percentage survival in open-pollinated progenies establishes the fact that survival is not distributed at random through the nursery but is definitely associated with paired progenies of a given parental plant.

As used in this study, the term "variability" refers to the morphological variations from the parental type found among the 2-year-old plants available for study. The aberrant plants showed numerous deviations from the parental type. Many were larger and more vigorous than the parent in all plant structures; many were much reduced in size and in vigor. The plants varied greatly in rhizome development, degree of spread, height, and leafiness, and in length, width, and color of leaf. It was found that panicle characters were good criteria of morphological variability. Panicles showed great variation in over-all size and shape, size of spikelets, number of spikelets, and color.

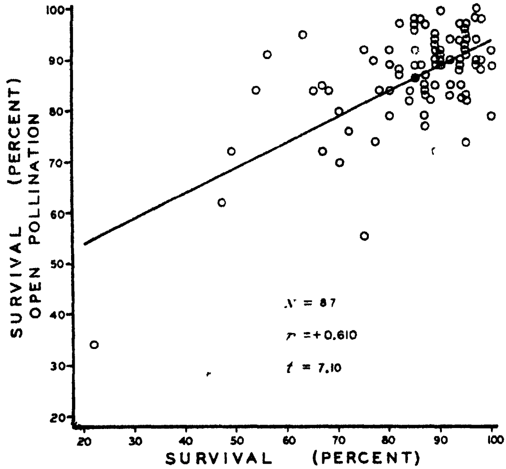

FIGURE 3.—Correlation of survival in progenies from self-pollination with survival in progenies from open pollination.

All the morphological variations just mentioned are grouped under variability, a term that the writer and other workers in this field, notably Müntzing (*19*, *22*), Åkerberg (*4*), and Tinney and Aamodt (*36*), assume to be an expression of sexuality existing in parental plants. Conservatism was exercised in classifying the plants in the categories of variant (sexual) and apomictic. No plant was classified as variant unless all evidence indicated the variability to be due unmistakably to genetic causes.

The correlation between the percentage variability in self-pollinated and open-pollinated progenies is highly significant (fig. 4).

indicating a genetic behavior inherent in any given parental plant. This correlation is greatly increased by the removal of three widely divergent pairs of observations. The correlation value becomes +0.818 (fig. 4). The observations that are divergent may possibly indicate plants whose behavior is significantly different from that of the main body of plants, which show a good fit with the regression line.

The distribution of the values for variability in 87 self-pollinated and 87 open-pollinated progenies is shown in figure 2, *B*. In order to determine whether significance could be ascribed to any differences existing between paired progenies from each parental plant, chi-square determinations were made and plotted against the theoretical distribution for $N=87$. The comparison indicates a discernible drift toward the region of high values. There are 12 values beyond the 5-percent point in the actual chi-square distribution, whereas 4.34 values beyond the 5-percent point are theoretically expected by chance alone. The question arises whether the increase over expectation represents plants that give self-pollinated progenies significantly different in variability from the open-pollinated progenies, or whether the increase results from chance. The data presented in figure 4 indicate that, of the 87 parental plants tested, 3 produced self-pollinated and open-pollinated progenies showing highly significant differences in morphological variability. In 2 instances, the plants of the open-pollinated progeny were more variable than those of the self-pollinated progeny; in 1, the reverse was true. However, results with by far the greater number of plants tested, 84 out of 87, show no indication of significant differences between the morphological variation found in self-pollinated progenies and that found in open-pollinated progenies.

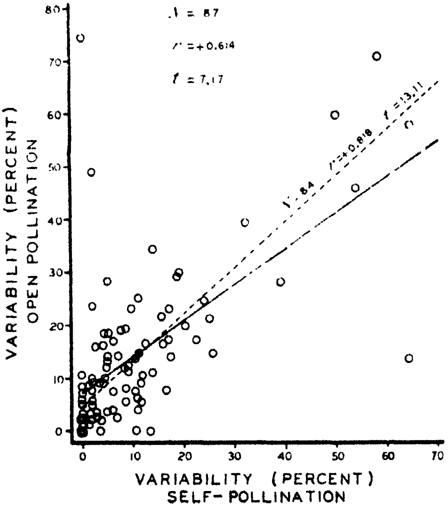

FIGURE 4.—Correlation of variability in progenies from self-pollination with variability in progenies from open pollination.

ANALYSIS OF ALL PROGENIES

In addition to the 87 plants discussed in the foregoing section, there were 28 parental plants which, because of failure of seed set under bag, gave only open-pollinated progenies. Since the previous analysis has suggested that, in general, no statistically significant differences exist between self-pollinated and open-pollinated progenies, those from each parent plant have been combined, giving an average population of 100.0 ± 1.6 for each plant. The total number of plants in the experimental nursery, upon which this study is based, was 10,066. This nursery contained offspring of 115 selected parental plants of Kentucky bluegrass, which were chosen as representing the range of morphological types found in the species. Table 4 presents the data on germination, polyembryony, survival, and variability obtained from these plants.

TABLE 4.—*Statistical data from progenies of 115 parental plants*

Item	Mean	Difference [1]	t	Lowest value	Highest value	Standard deviation
Germination:	*Percent*			*Percent*	*Percent*	
Combined progenies [2]	81.4±1.1	4.5±3.1	1.45	56.0	98.0	10.5
Open pollination [3]	76.9±2.9			32.0	99.0	15.2
All progenies [4]	80.3±1.1			32.0	99.0	12.0
Polyembryony:						
Combined progenies [2]	7.5± .7	1.9±1.2	1.58	0	31.6	6.2
Open pollination [3]	5.6±1.0			0	21.3	5.2
All progenies [4]	7.0± .7			0	31.6	7.0
Survival:						
Combined progenies [2]	85.9±1.1	1.6±2.5	.64	27.5	98.3	10.6
Open pollination [3]	87.5±2.2			53.5	100.0	11.8
All progenies [4]	86.3±1.0			27.5	100.0	10.4
Variability:						
Combined progenies [2]	12.9±1.4	7.6±3.04	[1] 2.50	0	65.5	13.3
Open pollination [3]	20.5±2.7			3.4	54.2	14.5
All progenies [4]	14.8±1.3			0	65.5	13.9
Size of progeny (number of plants):	*Number*			*Number*	*Number*	
Combined progenies [2] (8,697)	100.0±1.6			29	118	15.2
Open pollination [3] (1,369)	48.9±2.0			32	60	10.8
All progenies [4] (10,066) [5]						

[1] $P=0.05$ at $t=1.96$. $P=0.01$ at $t=2.58$.
[2] Progenies of 87 plants.
[3] Progenies of 28 plants.
[4] Progenies of 115 plants.
[5] Total number of plants in nursery.

The average percentage of occurrence of polyembryony in the seeds that germinated was 7.0 ± 0.7. Figure 5 gives the distribution of values for polyembryony in the 115 progenies. Only 4 plants failed to give twin seedlings. In the histogram the solid black bars indicate those progenies in which polyembryony was represented by only twin seedlings, the cross-hatched bars those progenies in which triple seedlings appeared in addition to twin seedlings. The highest value obtained for the frequency of triple seedlings was 4.3 percent, in a single open-pollinated progeny giving a value of 27.8 percent for polyembryony (twins plus triplets). As mentioned previously, the appearance of triple seedlings was invariably associated with a high incidence of polyembryony.

All available information gives an average percentage variability of 14.8 ± 1.3 for the progenies from the 115 selected parental plants of Kentucky bluegrass. The distribution of values is given in figure

6. Only 4 of the 115 plants gave progenies showing complete uniformity. A majority of the progenies (62) had variabilities between 0.1 percent and 12 percent. A smooth curve to fit the distribution should be expected to show a modal value of approximately 7 percent.

There is a difference of 7.6 between the variability in 87 parental plants that gave self-pollinated progenies (12.9 percent) and the variability in 28 parental plants that set no seed under bag (20.5 percent). This difference is statistically significant ($t=2.50$, $P<0.05$) and indicates a tendency for plants that are sterile under bag to be more sexual than other, more fertile plants in their mode of reproduction.

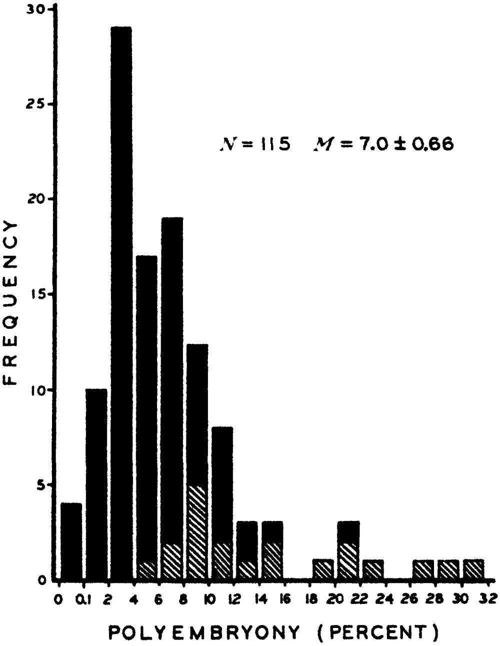

FIGURE 5.—Distribution of combined average values for polyembryony in self-pollinated and open-pollinated progenies from 115 plants of Kentucky bluegrass.

CORRELATION STUDIES

In these studies an attempt has been made to determine whether significant relationships exist between morphological variability in the offspring of a plant of Kentucky bluegrass and any one of several more easily and quickly determined criteria from the same plant. The analysis centers around variability, for this is the feature in which the plant breeder is ultimately most interested.

SOURCE OF MATERIAL, AND VARIABILITY

Figure 7 shows the distributions of variabilities found in the progenies of 115 parental plants of Kentucky bluegrass arranged on the basis of source of the parental stock. *A* shows the progenies of 16 plants from introductions and numbered strains; *B*, the progenies of 28 plants from pasture sources; and *C*, the progenies of 71 plants

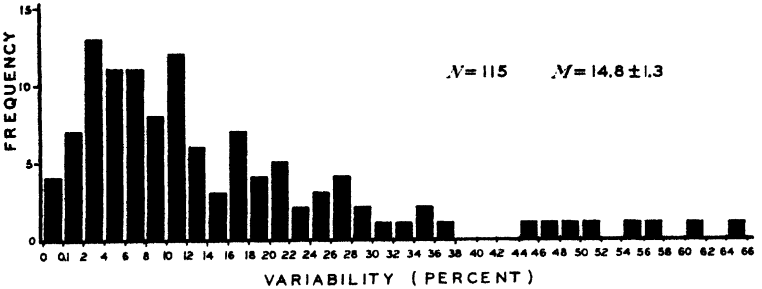

FIGURE 6.—Distribution of values for variability in progenies from 115 plants of Kentucky bluegrass.

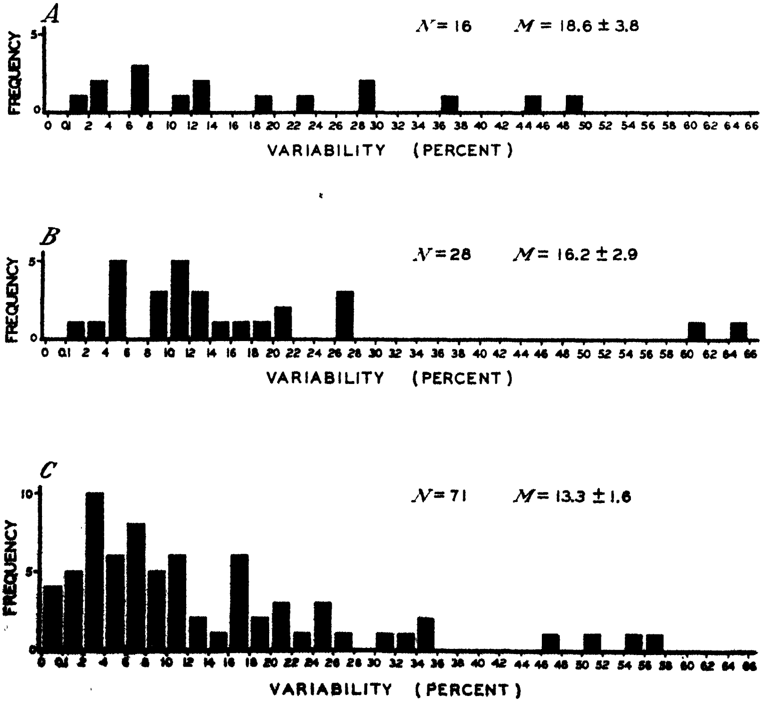

FIGURE 7.—Distribution of values for variability in progenies from (*A*) introductions and strains, (*B*) pasture sources, and (*C*) commercial seed sources.

from commercial seed sources. None of the differences between the averages for percentage variability is statistically significant ($t_{AB}=0.50$, $t_{BC}=0.88$, $t_{AC}=1.29$). There is, therefore, no evidence from this material that plants of Kentucky bluegrass from pasture sources and plants from commercial seed sources differ significantly in apomictic behavior, nor do plants from selected strains show very pronounced apomictic behavior in comparison with plants from other sources.

SEED SET UNDER BAG, AND VARIABILITY

As mentioned previously in the discussion of seed set under bag in the 115 selected parental plants, the 30-percent level of seed set apparently provides a convenient measure of a plant's ability to set seed under bag, with little distortion of results by environmental forces. Figure 8 shows the distribution of percentage variability in the progenies of plants separated on the basis of seed set under bag. *A* shows the progenies of plants that set less than 30 percent under bag; *B*, the progenies of plants that set more than 30 percent under bag. The difference of 4.9 percent between the averages for percentage variability is not statistically significant ($t=1.88$).

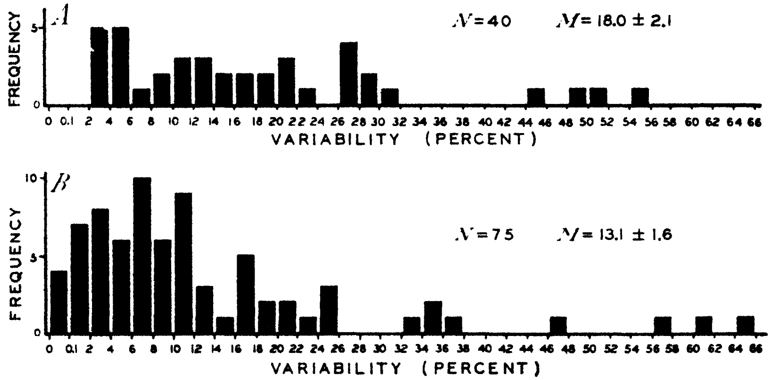

FIGURE 8.—Distribution of values for variability in progenies from (*A*) plants with less than 30 percent seed set under bag, and (*B*) plants with more than 30 percent seed set under bag.

CORRELATIONS OF VARIABILITY WITH GERMINATION, POLYEMBRYONY, AND SURVIVAL

Table 5 contains the tabulations of correlations between (1) germination and polyembryony, (2) germination and variability, (3) polyembryony and variability, and (4) survival and variability. Both the simple correlations and the second-degree partial correlations are given.

Column A contains the correlations in 87 self-pollinated progenies; column B, those in 87 open-pollinated progenies; and column C, those found after combining self-pollinated and open-pollinated progenies. Column D, headed "Corrected for attenuation," contains the correlation values calculated by the procedure suggested by Kemp (*17*), which makes use of paired values to eliminate systematic sampling errors. The values obtained by this procedure indicate the maximum degree of correlation existing in the material. Column E contains the correlation values obtained by using all available data from the 115 selected parental plants of Kentucky bluegrass. In the discussion that follows, reference will be made only to column E.

TABLE 5.—*Correlations of variability with germination, polyembryony, and survival in progenies of Kentucky bluegrass*

Items compared	(A) Self-pollinated progenies of 87 plants	(B) Open-pollinated progenies of 87 plants	(C) Combined progenies of 87 plants	(D) Corrected for attenuation (87 plants)	(E) All available data (115 plants)
Germination (G) and polyembryony (P):					
r_{GP}	+0.202	+0.130	[1] +0.210	[2] +0.310	[3] +0.222
$r_{GP.SV}$	+.206	+.140	+.203	[2] +.390	[3] +.202
Germination (G) and variability (V):					
r_{GV}	[1] −.225	−.099	[1] −.211	[2] −.386	[4] −.235
$r_{GV.SP}$	−.052	+.007	−.072	+.068	−.133
Polyembryony (P) and variability (V):					
r_{PV}	−.167	−.168	[1] −.210	[1] −.260	[3] −.205
$r_{PV.SG}$	−.173	−.177	[1] −.235	[3] −.391	[3] −.218
Survival (S) and variability (V):					
r_{SV}	[2] −.432	[1] −.265	[2] −.444	[2] −.645	[4] −.380
$r_{SV.PG}$	[2] −.401	[1] −.260	[2] −.432	[2] −.640	[4] −.378

[1] $P=0.05$ at $r=0.208$.

[2] $P=0.01$ at $r=0.270$.

[3] $P=0.05$ at $r=0.180$.

[4] $P=0.01$ at $r=0.235$.

There is a significant negative association between variability and polyembryony. The value for the simple correlation is −0.205 ($P<0.05$) (fig. 9). The value for the partial correlation, holding germination and survival constant, rises somewhat, to −0.218. Therefore, it may be said that in general there is a tendency for the higher percentages of morphological variability in the progeny of a plant to be associated with the lower percentages of polyembryony, and vice versa. This relation, however, cannot be stated positively, because of the barely significant values obtained for the correlation. A study of the character of the scatter diagram in figure 9 suggests the possibility that two groups may be present: (1) A group of plants whose progenies show a very high negative correlation between variability and polyembryony and (2) a group whose progenies show no association between these two characteristics. These selections of Kentucky bluegrass appear to be sufficiently diversified and heterogeneous to permit this supposition. The fact that the loss of plants was unusually high in some progenies has already been mentioned. The question thus arises as to whether practically all of the plants that failed to survive were not actually weak aberrant forms unable to survive under field conditions. The highly significant correlation value of −0.380 be-

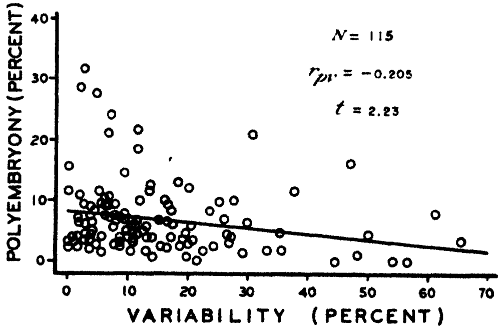

FIGURE 9.—Correlation of variability with polyembryony in progenies of 115 parental plants.

tween survival and variability suggests this (fig. 10). Holding germination and polyembryony constant, the value for the partial correlation is -0.378 ($P<0.01$). This degree of negative correlation between survival and variability implies a tendency for the most variable progenies to lose the most plants.

COMPARATIVE VARIABILITY OF PLANTS FROM SINGLE-EMBRYO SEED AND FROM POLYEMBRYO SEED

Facilities were not available for as complete an analysis of the twin and triple seedlings as was made of the single seedlings. It is, however, possible to obtain an average figure for the variability in the polyembryo material. Table 6 gives the complete analysis. Of the 290 triple and twin sets that contained plants differing morphologically, 73 sets (69 twin-seedling pairs and 4 triple-seedling groups) were put into the field and grown to maturity. At maturity, it was ascertained that 11 of the twin pairs consisted of identical plants. On the basis of this error in identification of aberrant pairs in the greenhouse, a correction factor of 84 percent was utilized. Thus, out of 1,441 plants from seed containing more than 1 embryo, 290 to 244 (84 percent of 290) may be considered aberrant. This calculation gives the value for variability in plants from twin and triple seedlings as 20.1 to 16.9 percent. The true value is in this range, with the greater likelihood that it is nearer 16.9 percent. This percentage is to be compared with the variability of 13.1 percent found in plants from seeds with a single embryo. The chi-square test indicates that great significance may be ascribed to this difference ($\chi^2=16.85$). Therefore it may be said that variability is significantly higher among plants derived from polyembryo seeds than from plants obtained

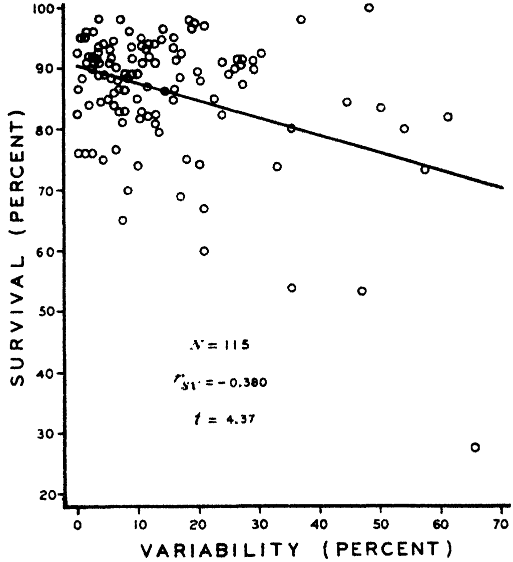

FIGURE 10.—Correlation of variability with survival in progenies of 115 parental plants.

from single-embryo seeds. But while the extent of morphological variation is significantly different between these 2 populations, the nature of this variation is the same. Plants of the same general morphological types may arise from either source.

TABLE 6.—*Comparative variabilities in plants from polyembryo seed and in plants from single-embryo seed* [1]

Kind of seed	Plants—				Individual plants—		Groups of plants—		Variability
	Appearing		Surviving for study		Surviving after germination	Aberrant	Discarded as identical	Dissimilar or questionable	
	Number	*Percent*	*Number*	*Percent*	*Number*	*Number*	*Number*	*Number*	*Percent*
Polyembryo	1,196	7.4	711	80.05	1,441	290([2] 244)	421	290	20.1([2] 16.9)
Triplets	39	.24	19	82.9	57	4	15	4	7.0
Twins	1,157	7.2	602	80.0	1,384	286	406	286	20.7([2] 17.3)
Single embryo	14,888	92.6	10,066	86.9	10,066	1,314			13.1

[1] Number of seeds started, 19,858; number germinated, 16,084; germination, 81.0 percent.

[2] Corrected value (see p. 241).

Where the components of twin seedlings and of triple seedlings were identical, the type of plant invariably conformed to that of the parent. Where triplets were dissimilar, one plant was larger and more vigorous and two plants were identical with the parent plant type. In the triple seedlings no aberrant plant was smaller or less vigorous than the parent plant, but this observation can hardly be significant in view of the small number of triplets (19) available for study. Where twin seedlings were dissimilar, the aberrant plant almost invariably appeared first as the smaller and slower growing seedling, although it generally became the larger and more vigorous plant of the pair. Therefore it would seem that the aberrant, or sexually produced, member of a dissimilar pair of seedlings usually comes from an embryo that for some reason is later in germinating than the apomictically produced embryo associated with it.

ABERRANT PLANTS IN PROGENIES

Representative parental plants, apomictically reproduced offspring, and aberrant sexually produced offspring were analyzed to determine the relative rate of polyembryony between parental plants and offspring and also the occurrence of albino seedlings, since a few of these had appeared in seed lots of the original material. Open-pollinated seed was germinated in Petri dishes in the manner described previously in connection with the experimental nursery.

The probable chromosomal complements of many of the aberrant plants were determined by an analysis of pollen-grain size. These results are presented and supplemented with actual chromosome counts in a number of instances to show the manner of origin of the aberrant plants.

POLYEMBRYONY

The results of an analysis of relative rates of polyembryony are presented in table 7. In each family *P* represents the parental plant; *A*, the apomictically reproduced type; and the other letters refer to plants of the progeny that deviated from the parent type in morphological characters and are presumed to have arisen by sexual processes. Determinations are shown on seeds from 39 plants (5 parental and 34 offspring). It was found that the percentage of polyembryony in 9 offspring plants was significantly different from that in the parents; all had significantly lower rates. (Two aberrant plants in progenies not included in the table had rates of polyembryony significantly higher than those of the parent plants.) In only 1 instance did the parent and the apomictic type differ significantly in rate of polyembryony, but germination in the latter was poor and sampling errors may account for the observed difference. The value of χ^2 (4.77) is barely beyond the 5-percent point.

TABLE 7.—*Pollen-grain size, chromosome number, polyembryony, and albino seedlings in selected plants of Kentucky bluegrass and their progenies*

Illustration and plant-pedigree No.	Pollen-grain size	Chromosome number	Total seed germinated	Polyembryony				Albino seedlings
				Triplets	Twins	Total		
						Number	Percent[1]	
Figure 11:	*μ*		*Number*	*Sets*	*Pairs*	*Number*	*Percent*	*Number*
P, 37-KB 1 (11), parent		±56	279	0	13	13	4.7	[2] 2
A, 38-KB 3 (8), type		56	99	0	0	0	[3] 0	2
B, 38-KB 3 (6)		±48	0	0	0	0		
C, 38-KB 3 (9)		(4)	0	0	0	0		
D, 38-KB 4 (4)		±50	30	0	1	1	3.3	
E, 38-KB 4 (7)		±56	234	0	6	6	2.6	4
F, 38-KB 4 (3)		±58	252	0	0	0	[5] 0	
Figures 12 and 13.								
P, 37-KB 140 (11), parent	[6] 25.9±0.3	±45	303	0	7	7	2.3	
A, 38-KB 130 (4), type	25.2± .2	±45	439	0	7	7	1.5	
B, 38-KB 130 (8)	[5] 34.8± .6	±62	207	0	2	2	.9	
C, 38-KB 130 (21)	[5] 33.4± .5	±70	145	0	0	0	0	
D, 38-KB 130 (13)	[5] 33.8± .4	±66	229	0	5	5	2.1	
E, 38-KB 130 (26)	[5] 32.2± .6	±67	256	0	1	1	.3	
F, 38-KB 130 (1)	[5] 32.4± .6	±60	199	0	7	7	3.5	
No figure:								
37-KB 172 (14), parent		±80	90	1	21	22	24.4	
38-KB 195 (1), type			157	1	39	40	25.5	
38-KB 195 (21)		±42	12	0	0	0	(?)	
38-KB 196 (1), type			171	4	44	48	25.7	
38-KB 196 (5)		110-120	182	0	17	17	[5] 9.3	
Figures 14 and 15:								
P, 37-KB 175 (14), parent	[6] 29.0± .7	±50	213	1	18	19	8.9	1
A, 38-KB 206 (16), type		±50	382	0	30	30	7.8	
B, 38-KB 206 (1)		34	0	0	0	0		
C, 38-KB 206 (11)		(7)	43	0	0	0	0	
D, 38-KB 206 (4)		(7)	99	0	3	3	3.0	
E, 38-KB 206 (8)	[5] 17.5± .3	(4)	2	0	0	0		
F, 38-KB 206 (6)	30.0± .7	(7)	186	0	16	16	8.6	
G, 38-KB 206 (28)		(7)	185	0	2	2	[5] 1.1	
H, 38-KB 205 (6)	[5] 25.9± .6	(7)	175	2	22	24	13.9	
I, 38-KB 205 (14)	30.5± .4	(7)	108	0	1	1	[5] .9	
B_1, 38-KB 206 (62)-1	[5] 21.8± .4	±48	0	0	0	0		
C_1, 38-KB 206 (63)-1	[5] 25.4± .5	(4)	199	0	1	1	[5] .5	
C_2, 38-KB 206 (63)-2, type	30.7± .4	±50	303	0	37	37	12.2	[8] 6
Figure 16:								
P, 37-KB 135 (131), parent	[6] 29.5±1.0	42	242	0	10	10	4.1	
A, 38-KB 118 (40), type	28.1±1.4	42	216	0	4	4	1.9	
B, 38-KB 118 (47)		(4)	195	0	15	15	7.7	
C, 38-KB 118 (42)		±42	172	0	1	1	[5] .6	
D, 38-KB 118 (36)		±45	170	0	0	0	[3] 0	
E, 38-KB 118 (39)	[5] 22.3± .3	±40	128	0	9	9	7.0	
F, 38-KB 118 (46)	[5] 34.6± .5	±75	165	0	2	2	1.2	

[1] Based on total number of seeds germinated.
[2] 1 albino single. 1 pair of twins: 1 member albino; 3 members green.
[3] $P < 0.05$.
[4] No material.
[5] $P < 0.01$.
[6] Standard of reference.
[7] Diploid.
[8] 3 albino singles. 1 pair of twins: 1 member albino; 1 member green. 1 pair of twins, both albino.

The points of interest in connection with these studies on relative rates of polyembryony are as follows:

(1) Apomictically produced plants show the same rates of polyembryony as their respective parents, indicating a heritable control of the production of polyembryonic seed.

(2) Of the 58 aberrant plants tested, 47 did not differ significantly from their parents in rate of polyembryony, 2 had a significantly higher rate, and 9 a significantly lower rate. There is no association between plant type or manner of origin and rate of polyembryony.

ALBINO SEEDLINGS

Albino seedlings have appeared in progenies of two plants.

Selection 37-KB 1 (11).—This plant and its offspring are discussed in a later section. Evidence indicates that it is perhaps the most sexually reproduced of the selected material. In 279 germinated seeds of the plant shown in figure 11, *P*, there appeared 1 albino single seedling and 1 albino seedling associated with a green seedling from a twin-embryo seed. Two progenies from its offspring have contained albinos. One was the progeny from an apomictically reproduced member of the population (see fig. 11, *A*), which gave 2 albino single seedlings in 99 germinated seed; the other was an aberrant plant from the same population (see fig. 11, *E*), which gave 4 albino single seedlings in 234 germinated seed.

Selection 37-KB 175 (14).—This plant and its progenies are also described in a later section. In 213 seedlings from the parent plant shown in figure 14, *P*, there appeared 2 albino seedlings. An apomictically reproduced plant of its progeny has given a surprisingly large number of albinos. (See fig. 14, C_2) This plant was associated with C_1 in a pair from a twin-embryo seed. In 303 germinated seeds, there were 3 albino single seedlings, 1 albino seedling that occurred with a green seedling to comprise a pair of twins, and 2 albino seedlings that occurred together as twin seedlings. No albinos have appeared in seedlings from any aberrant plants obtained from this parent.

CYTOLOGICAL STUDIES

Pollen measurements.—Mature pollen grains were mounted in acetocarmine, and their diameters measured by means of a filar micrometer. The number of grains measured per plant ranged from 20 to 25. The small number seems entirely adequate, judging by the sizes of the standard errors, which are decreased, on the average, only about 0.3μ when $N = 100$.

The measurement of pollen grains is useful in determining gross chromosomal changes, since, as has been found in numerous plants, pollen-grain size is proportional to chromosome number. This has been applied to *Poa* by Müntzing (*22*) and the writer (*7*). The results are presented in table 7 and require little individual comment. The fact is perfectly clear that the pollen of many of the aberrant plants is significantly different in size and may be assumed to reflect the chromosomal complement of the plant. In no case did the size of the pollen of the apomictically reproduced progeny differ significantly from that of the parental plant. However, the aberrant plants, on the basis of pollen-grain size, may be placed in one of three categories: (1) Pollen significantly smaller than the parent, indicating lower chromosome numbers; (2) pollen significantly larger, indicating plants with a great increase in chromosome number, probably "triploid"; and (3) pollen not significantly different, indicating chromosome numbers so near that of the parent that the pollen differences did not give statistically significant values; these aberrant plants are "diploids by amphimixis."

Chromosome counts.—The somatic chromosome number was obtained from root-tip divisions. The material was killed and fixed in the modification of Navashin's fluid proposed for *Poa* by Müntzing (*19*). The material was dehydrated and embedded by the procedure devised by Randolph (*29*), sectioned at 12μ, and stained by the modified crystal-violet-iodine technique proposed by Smith (*31*).

The extent and nature of chromosomal phenomena in *Poa pratensis* have been analyzed in very detailed studies by Müntzing (*19, 20, 21, 22*), Armstrong (*6*), and Åkerberg (*4*). Therefore it has not been thought necessary in this study to do more than determine the manner of origin of the aberrant or sexually produced plants that have appeared in the writer's material. Some indications of their probable chromosomal complements have already been obtained from a study of morphological features and pollen-grain size.

The results of the cytological study are tabulated in table 7. It is seen that by far the greatest number of the aberrant plants are to be considered as arising through the fertilization of reduced egg cells by reduced pollen grains, since their chromosome numbers do not deviate markedly from the chromosome number found in the parent plant. Two "haploid" individuals have been identified, one of which is shown in figure 14, *B*. Triploid aberrant plants have been identified with certainty in 12 instances. These plants are usually of increased vigor, but in 2 instances the triploid plants were decidedly less vigorous than the parental plant. This might indicate that chromosomal multiplication in *Poa* is subordinate to genic constitution in the determination of plant vigor. Of considerable interest is the progeny shown in figures 12 and 13, where every aberrant plant studied has been demonstrated to be of triploid origin. On the other hand, in the progeny of the most highly sexual plant studied (see fig. 11), all aberrant plants presumably have arisen by the union of reduced egg and sperm; that is, they are "diploid by amphimixis."

Only two pairs of twin seedlings have been studied cytologically. In each instance, the aberrant plant has been shown to be of probable diploid origin. The aberrant plant of a group of triple seedlings has been shown to be a triploid.

To summarize the results of the cytological studies, we may enumerate the following points:

(1) The aberrant plants that have appeared in the progenies of Kentucky bluegrass may be classified according to their manner of origin as haploids, sexually produced diploids, or triploids. The diploids have been by far the most prevalent aberrant type. The haploid individual either is of infrequent origin or fails to survive.

(2) Pollen-grain size is of value in identifying certain types of aberrant plants but must be used with caution when applied to the identification of others. Pollen size may be used with complete reliability, for example, in identifying plants of triploid origin and sexually produced diploids. Its use in presuming a plant to be of

haploid origin if its pollen is significantly smaller than that of the parent plant is not reliable, since many aberrant plants with extremely small pollen grains have been shown to have chromosome numbers not greatly different from the parental type. Presumably, if the aberrant plants are of low vigor, as many are, physiological factors, such as rate of growth and efficiency of nutrition, may cause the formation of smaller cells than should theoretically be produced on the basis of the plant's chromosomal complement.

(3) Similar chromosomal conditions are present in aberrant plants whether produced from seed with multiple embryos or from seed with a single embryo. There is thus no essential difference in their manner of origin.

DESCRIPTION OF REPRESENTATIVE PROGENIES

SELECTION 37–KB 1 (11)

Plant 37–KB 1 (11) (fig. 11) was obtained in a progeny grown from seed collected in a West Virginia pasture. It was of moderate vigor and spread, fairly leafy, and not very coarse. It flowered profusely in the greenhouse, where it set seed well under bag. Subsequent bagging in the field showed fertility to be quite high, well over 60 percent seed being set under bag.

Self-pollinated progeny 38–KB 3.—Germination of seed, 64.0 percent; polyembryony, 3.1 percent. Survival of plants was the lowest observed (21.5 percent), only 12 of the original 56 plants persisting. Of these 12 surviving plants, 7 differed from the parental type, a variability of 58.3 percent. Three of the 7 were similar, being much larger and coarser than the parent, and very stemmy (fig. 11, *F*). The other 4 were less vigorous than the parent. One (*B*) was an exceedingly small plant, having short, narrow, dark-green leaves and few culms, one-third as tall as the parent and with extremely small panicles; another (*C*) was very erect in its growth, had little spreading ability, and its leaves were narrow, wiry, and light green. The other 2 of the aberrant plants, that were less vigorous than the parental type, though not so extreme as the 2 just described, differed unmistakably from the parent.

Open-pollinated progeny 38–KB 4.—Germination, 56.0 percent; polyembryony, 3.8 percent. Survival was very low (34.0 percent), only 17 remaining out of an original 50 plants. Fourteen of these plants differed from the parental type, a variability of 70.6 percent, the highest obtained. Eight of these were more vigorous; 7 were similar and corresponded in type of plant to the group of 3 already described in the selfed progeny (fig. 11, *F*). The other, more vigorous plant was not so tall but was a vigorous spreader. Its leaves were more numerous and were dark green (fig. 11, *E*). The other aberrant plants were smaller and less vigorous than the parent. One is shown in figure 11, *D*.

No complete set of twin seedlings survived for study, and, in view of the high rates of loss of plants and variability and the high correlation between them, the conclusion may be reached that all twin pairs had dissimilar members. The aberrant plants were presumably too weak to survive.

These progenies of a plant obtained from a pasture source have been described in some detail because they present certain extremes in

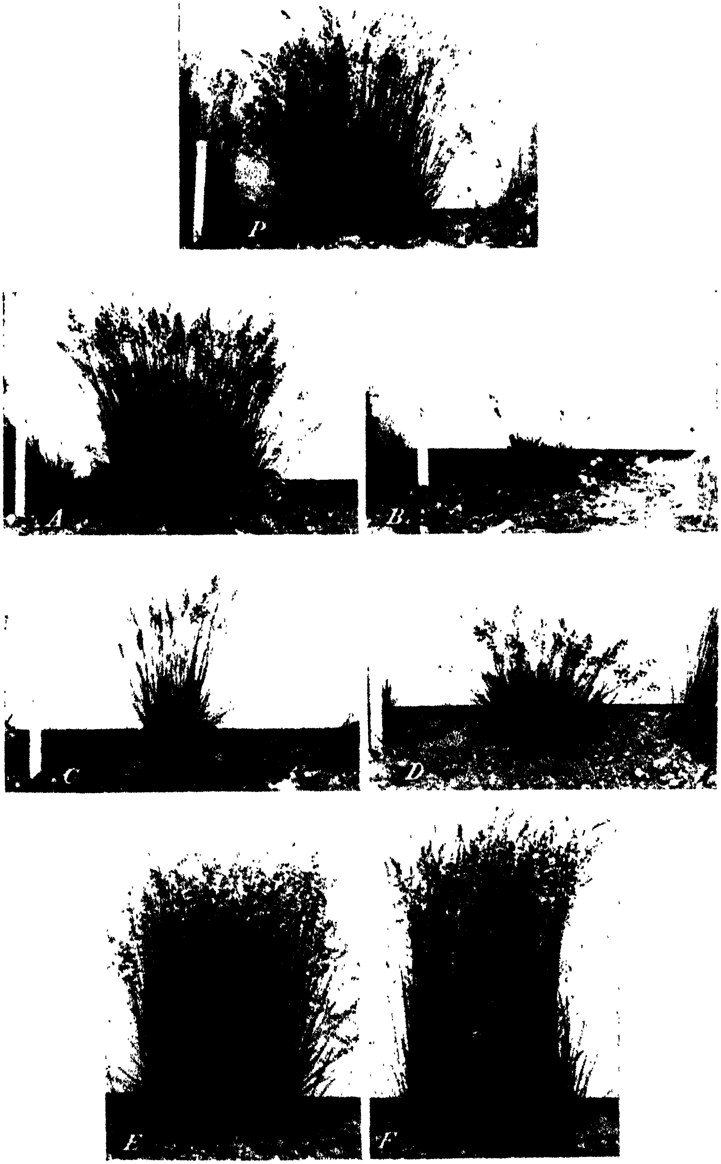

FIGURE 11.—*P*, Kentucky bluegrass parent plant 37–KB 1 (11), showing mature field habit. *A*, An apomictically reproduced matroclinous plant, 38–KB 3 (8), of its progeny. *B–F*, Five aberrant plants of its progeny: *B* 38–KB 3 (6); *C*, 38–KB 3 (9); *D*, 38–KB 4 (4); *E*, 38–KB 4 (7); and *F*, 38–KB 4 (3). Plants *A*, *B*, and *C* occurred in the progeny from self-pollination; plants *D*, *E*, and *F*, in the progeny from open pollination. *P* and *A* have the same chromosome number; counts on *B*, *D*, *E*, and *F* indicate they are "diploids by amphimixis." No information is available for plant *C*.

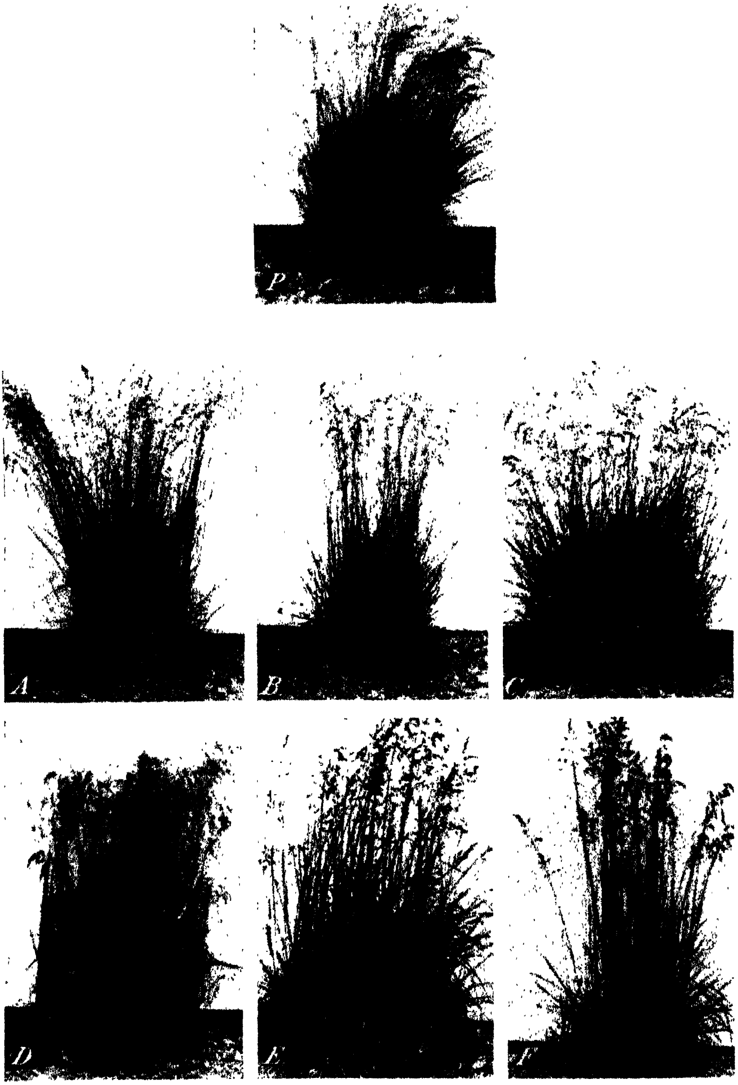

FIGURE 12.—*P*, Kentucky bluegrass parent plant 37–KB 140 (11), showing mature field habit. *A*, An apomictically reproduced matroclinous plant, 38–KB 130 (4), of its progeny. *B–F*, Five aberrant plants of its open-pollinated progeny: *B*, 38–KB 130 (8); *C*, 38–KB 130 (21); *D*, 38–KB 130 (13); *E*, 38–KB 130 (26); and *F*, 38–KB 130 (1). No self-pollinated progeny was available. Plants *P* and *A* have the same chromosome number; counts on *B*, *C*, *D*, *E*, and *F* indicate all are triploids and presumably arose through the fertilization of unreduced eggs by reduced pollen.

behavior and some rather interesting relationships. The points of interest are (1) good seed set under bag; (2) poor germination, 60 percent; (3) a low value for polyembryony, 3.3 percent; (4) the lowest value obtained for survival, 27.5 percent; and (5) the highest value obtained for morphological variation, 65.5 percent. It has been shown that aberrant plants in the progeny may show a wide range of types, that these may be either more vigorous or less vigorous than the parental type, and that similar aberrant plant types are obtained in self-pollinated and open-pollinated progenies.

SELECTION 37–KB 140 (11)

Plant 37–KB 140 (11)(figs. 12 and 13) was selected in a progeny from commercial seed grown in Kentucky. It was in no way representative of the plants from commercial seed but is presented as one of the most unusual plants of Kentucky bluegrass in the selected material. Leaves were numerous, light green in color, and were the narrowest found in the nursery from which selections were made. . The plant had also a decidedly different type of growth. Rhizomes were unusually poorly developed, and the plant assumed the appearance of a "bunch" grass (fig. 12, *P* and *A*). Panicles were delicate in texture and spikelets extremely small (fig. 13, *P* and *A*). This plant has never flowered in the greenhouse. It has been entirely sterile under conditions of bagging in the field. Seed set was poor on open pollination.

Open-pollinated progeny 38–KB 130.—Germination, 76 percent; polyembrony, 3.6 percent; survival of plants in the field, 90.5 percent; variability, 27.1 percent; 13 of 48 plants were aberrant. These plants differed from most groups of aberrant plants in that none of them was smaller or less vigorous than the parental plant. No 2 were quite alike, but instead presented a gradual range of types from the plant shown in figures 12, *B*, and 13, *B*, which had a type of growth similar to the parent plant but which was darker green and had somewhat wider leaves, to the extreme type shown in figures 12, *E*, and 13, *E*, which had an entirely different habit of growth and plant characters. This plant was vigorous and spreading, with deep-green leaves, unusually broad and thick, and panicles and spikelets much larger and heavier than those of the parent.

No twin seedlings of this plant were available for study.

SELECTION 37–KB 172 (14)

Plant 37–KB 172 (14) was obtained in a uniform progeny grown from seed of the strain of Kentucky bluegrass Ottawa 994 and also Minnesota P-37. It was unusually high in vigor, with good spread and with leaves numerous, broad, and the longest found in the nursery.

No flowering occurred under greenhouse conditions. In the field, the plant was later in its blooming date than the bulk of Kentucky bluegrass material and produced comparatively few panicles. Seed set under bag was poor, less than 30 percent.

Self-pollinated progeny 38–KB 195.—Germination, 59 percent; polyembryony high, 20.3 percent; survival in the field, 68 percent; variability, 3.1 percent. The progeny was remarkably uniform, conforming exactly to the parental type plant. Out of 32 plants, only 1 aberrant plant was found; this was very small, with no spread; the

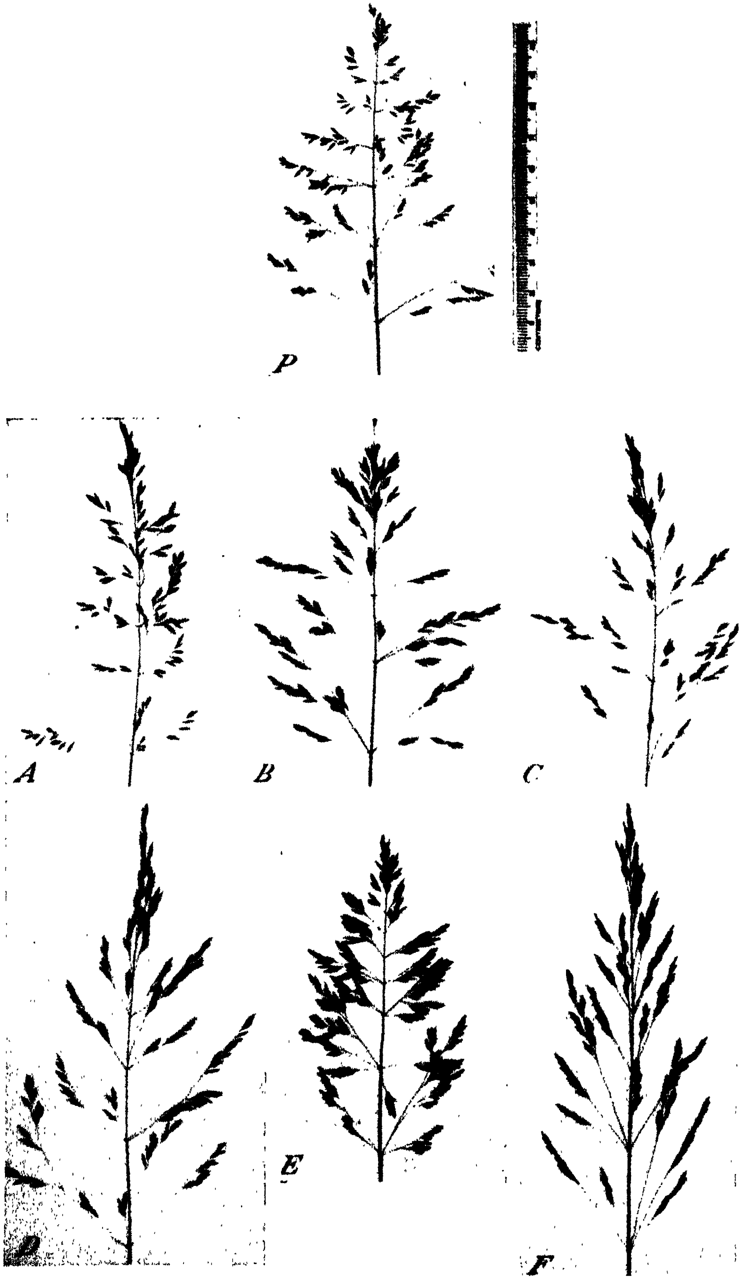

Figure 13.—Mature panicle characters of the plants shown and described in figure 12.

leaves were short, wiry, and light green; the culms reached only one-third the height of the parent, and the panicles were short and very small. Chromosome counts showed this plant to be haploid.

Open-pollinated progeny 38–KB 196.—Germination, 83 percent; polyembryony the highest found (36.1 percent), with a high incidence of triple seedlings (2.4 percent); survival, 83.5 percent; variability, 2.4 percent. The open-pollinated progeny was also uniform, except for 1 plant in the population of 41. This differed from the parental type in being more leafy, with leaves wider and darker green, culms not so tall as in the parent, but panicles significantly larger and heavier. Chromosome counts indicate this plant to be triploid.

Forty-two pairs of twin seedlings were obtained from 142 germinated seed of this plant. Of these, only 26 pairs survived for study, 4 of which were dissimilar. Of these, 1 aberrant plant showed greater vigor and larger foliage than its twin; the other 3 were smaller than their respective apomictic twins. This represents a variability in the surviving plants from twin seedlings of 7.9 percent. No triple seedlings survived as intact groups of 3 available for study.

This parental plant and its progenies are presented to establish the following points of interest: (1) Poor seed set under bag; (2) comparatively poor germination, 71.0 percent; (3) an unusually high value for polyembryony, 31.6 percent, the highest obtained; and (4) practically complete conformity to the parental type in the progenies, variability being 2.7 percent (2 plants out of 73).

SELECTION 37–KB 175 (14)

Plant 37–KB 175 (14) (figs. 14 and 15) was selected in a progeny grown from seed of a strain (O. A. C. No. 1) of Kentucky bluegrass received from the Ontario Agricultural College. It was a bunchy, compact plant; the leaves were short, broad, and dark green; the culms hardly taller than the leaves; the panicles compact, thick, very broad in relation to their length; and the spikelets exceedingly large.

This selection failed to flower in the greenhouse. In the field, it set seed only moderately well under bag, about 50 percent. Plants of this type were the latest of any Kentucky bluegrass to flower in the field, being at least a week to 10 days later than all other plants of the species.

Self-pollinated progeny 38–KB 205.—Germination, 86.0 percent; polyembryony, 10.5 percent; survival in the field, 90.0 percent; variability, 18.5 percent. Ten plants out of 54 were aberrant; the others conformed perfectly to the parental type. As a general rule, all plants that did not conform to the parent type were smaller and less vigorous. A wide range of plant types was present. Four of the 10 aberrant plants were of the same type, being extremely small and weak, barely able to survive, and producing only 2 or 3 culms (figs. 14 and 15, *C*).

Open-pollinated progeny 38–KB 206.—Germination high, 94.0 percent; polyembryony, 6.4 percent; survival, 91.5 percent; variability, 29.1 percent. Sixteen of the 55 plants were aberrant; the others resembled the parental type perfectly. As was the case in the self-pollinated progeny, all of the aberrant plants were less vigorous than the parent plant. Many morphological types were represented. It

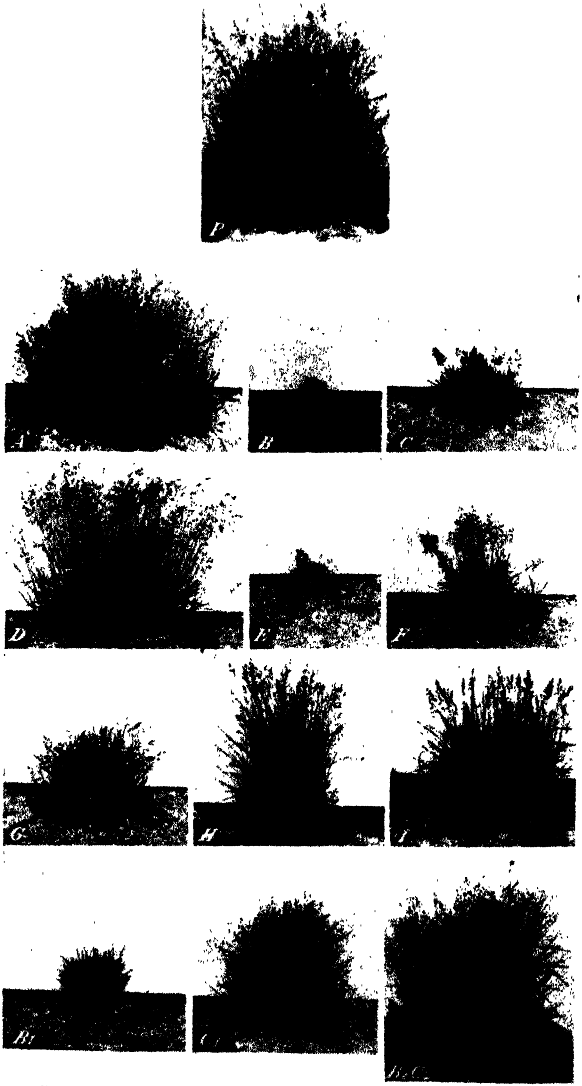

FIGURE 14.—*P*, Kentucky bluegrass parent plant 37–KB 175 (14), showing mature field habit. *A* and B_2C_2, Two apomictically reproduced matroclinous plants of its progeny: *A*, 38–KB 206 (16); and B_2C_2, 38–KB 206 (63)–2. *B–I*, Eight aberrant plants of its progeny from single-seedling sources: *B*, 38–KB 206 (1); *C*, 38–KB 206 (11); *D*, 38–KB 206 (4); *E*, 38–KB 206 (8); *F*, 38–KB 206 (6); *G*, 38–KB 206 (28); *H*, 38–KB 205 (6); and *I*, 38–KB 205 (14). B_1 and C_1, Two aberrant plants of its progeny from twin-seedling sources: B_1, 38–KB 206 (62)–1; and C_1, 38–KB 206 (63)–1. Plants *A* to *G*, B_1, C_1, and B_2C_2 occurred in the progeny from open pollination; plants *H* and *I*, in the progeny from self-pollination. *P*, *A*, and B_2C_2 have the same chromosome number; counts indicate *B* to be "haploid"; and *C*, *D*, *F*, *G*, *H*, *I*, and B_1, "diploids by amphimixis." No information is available for plants *E* and C_1.

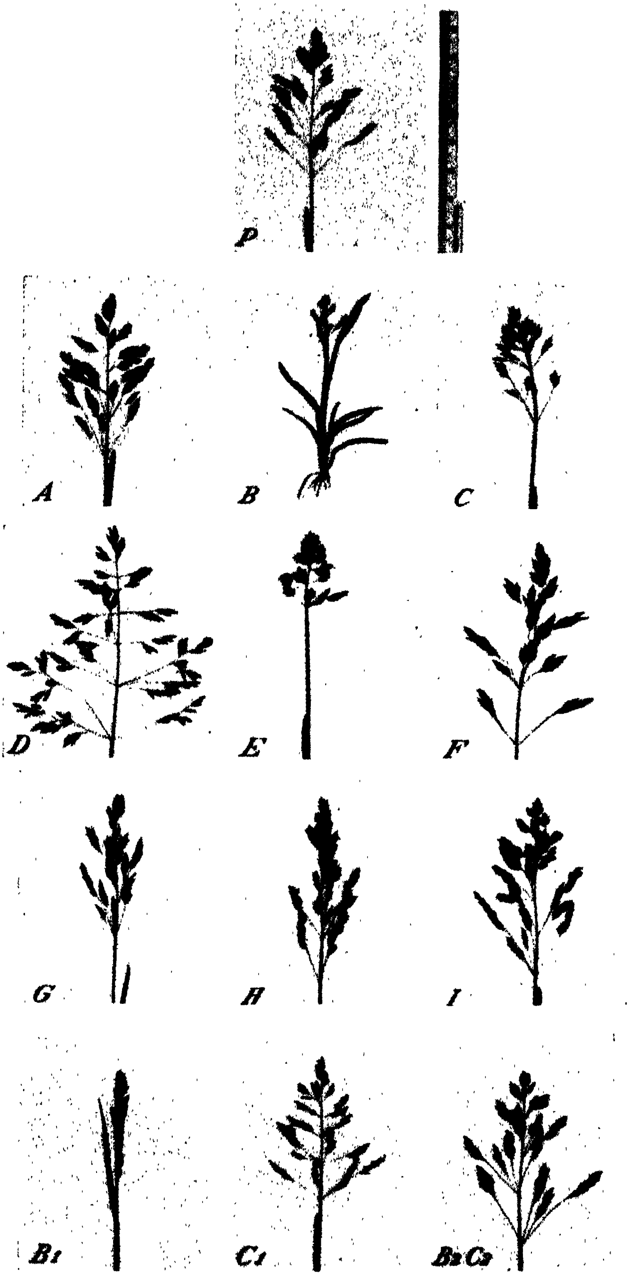

FIGURE 15.—Mature panicle characters of the plants shown and described in figure 14.

was possible, however, to group many of the variant plants into groups having more or less the same general growth characteristics. Thus, there were 5 plants which were similar to the 2 shown in figures 14 and 15, *B* and *E*; 3 similar to the one shown in *C*; and 5 of the same general type as the one shown in *G*. The remaining aberrant plants conformed to none of the above groupings. Two interesting departures from the parental type are illustrated in figures 14 and 15, *D* and *H*. The plant shown in *D* had shorter, narrower, lighter green leaves; the culms exceeded the leaves and the panicle was much larger and of a more open type.

Ten of the 15 pairs of twin seedlings of this plant survived for study. Six pairs were dissimilar, giving a value for variability of 30 percent in the seedlings from polyembryo seeds. Without exception, these aberrant members of the pair were less vigorous than the apomictic type and differed strikingly from it. Two pairs of twin seedlings from this plant are shown in figures 14 and 15, B_1, C_1, and B_2 C_2, the last mentioned being the apomictically reproduced member and identical in each pair of twin seedlings. Attention is called here to the demonstration that the same plant types may be found in both progenies from seed with a single embryo and progenies from polyembryo seed.

SELECTION 37-KB 135 (131)

Plant 37-KB 135 (131) (fig. 16) was chosen from a progeny grown from commercial seed from Kentucky. It was rather distinctive in vegetative characters. As it appeared in the nursery of spaced plants, it was one of the most leafy and aggressive types found, spreading extensively and forming a loose sod. Leaves were of moderate length and width and very soft. This plant flowered profusely in the greenhouse. Seed set under bag was less than 30 percent under both greenhouse and field conditions.

Self-pollinated progeny 38-KB 117.—Germination, 79.0 percent; polyembryony, 5.1 percent; survival under field conditions, 83.5 percent; variability, 54.0 percent. Of the population of 50 plants, only 23 resembled the parental type (fig. 16, *P* and *A*). A surprising feature of the plants differing from the parent type was that 23 of the 27 aberrant plants showed unmistakably the same morphological features. This group bore no resemblance to the parent. The plants were quite erect, very coarse and stemmy; leaves were longer, wider, and not as soft as the parental type. The plant illustrated in figure 16, *F*, shows the general morphological features of the group of 23 aberrant plants. Of the 4 remaining variant plants, 1 resembled the type described above but had significantly wider leaves; 2 were alike in general features, showing poor spreading qualities and few, narrow, light green leaves (fig. 16, *B*); and one was a small low-growing plant, with short, soft, wide, dark-green leaves (fig. 16, *E*).

Open-pollinated progeny, 38-KB 118.—Germination, 82.0 percent; polyembryony, 3.6 percent; survival, 83.5 percent; variability, 46.0 percent. Twenty-seven of the 50 plants in the progeny resembled the parental plant. The aberrant plants showed a greater diversity of types than did the plants of the selfed progeny, but the greatest number of them showed the features already described for the group illustrated by the plant in figure 16, *F*. There were 11 plants of this type. The remaining 12 aberrant plants were practically individual in their features. Four of the widely deviating plants are shown in

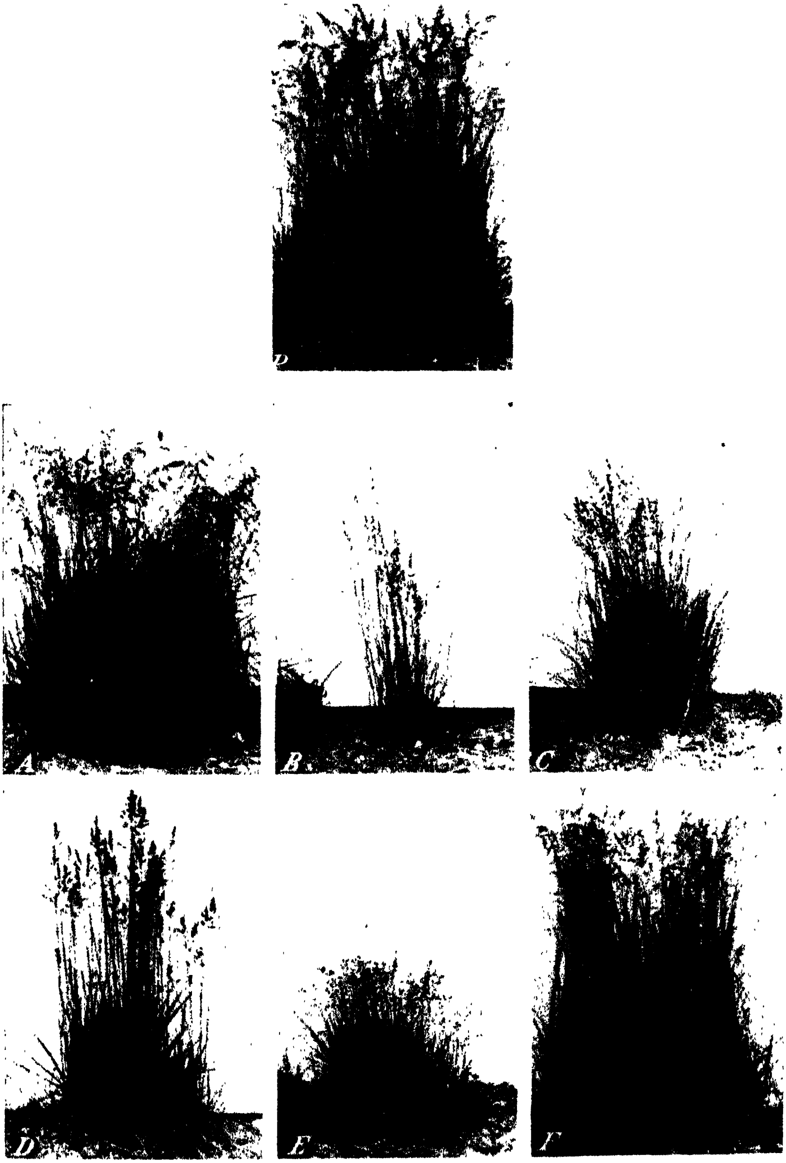

FIGURE 16.—*P*, Kentucky bluegrass parent plant 37–KB 135 (131), showing mature field habit. *A*, An apomictically reproduced matroclinous plant, 38–KB 118 (40), of its progeny *B–F*, Five aberrant plants of its progeny: *B*, 38–KB 118 (47); *C*, 38–KB 118 (42); *D*, 38–KB 118 (36); *E*, 38–KB 118 (39); and *F*, 38–KB 118 (46). All plants occurred in the progeny from open pollination. Plants *P* and *A* have the same chromosome number; counts indicate plants *C*, *D*, and *E* to be "diploid by amphimixis"; plant *F* is "triploid." No information is available for plant *B*.

figure 16, *B*, *C*, *D*, and *E*. The one shown in *E* was the most striking variation in the progeny. Leaves were numerous, very soft, short, narrow, and deep green; panicles were quite small and delicate.

All twin seedlings from this parental plant survived. Of seven pairs, four were dissimilar, giving a value for variability of 28.5 percent among plants from polyembryo seeds. All aberrant plants from twin sources were smaller and less vigorous than the apomictic member of the pair.

DISCUSSION

STERILITY AND FERTILITY IN POA PRATENSIS

Seed set under bag is not the ideal measure of self-fertility, since the introduction of physiological factors such as altered aeration, light, and moisture relationships may result in the determination, not of the plant's inherent fertility upon isolation, but only of its ability to set seed under conditions of bagging. However, with all the disadvantages attending the use of bags, Nilsson's work (*25*) and the results of the writer show clearly that plants of Kentucky bluegrass differ markedly in their ability to set seed under bag and these observations indicate a genotypical basis for sterility and fertility. These conclusions, both by Nilsson and the writer, have been based on replicated observations accumulated over several years and obtained by the use of replicated bags. Nevertheless, the results must be used with caution. There is no a priori reason for assuming that a plant which sets no seed under bag will also be sterile when unbagged but isolated from foreign pollen. This fact may have important practical implications if the breeding program should center about such methods as hybridization and strain building.

ANALYSIS OF SELF-POLLINATED AND OPEN-POLLINATED PROGENIES

The measurements made on germination and polyembryony have indicated that no statistical significance may be ascribed to differences between self- and open-pollinated progenies of the same plant. Data on polyembryony are particularly striking in this respect. Plants vary tremendously in their inherent rates, but rate is apparently little affected, if at all, by the type of pollination or by environmental factors encountered in this study. This lack of environmental effect is understandable if it be assumed that apomixis and its attendant phenomena are genic in character.

In the case of morphological variability the relationship is less clear, but it may be said with a fair degree of certainty that most of the plants give self- and open-pollinated progenies which do not differ significantly in variability. Figures 2, *B*, and 4 and table 3 illustrate this similarity. However, there are some paired progenies that differ markedly in variability, which indicates a fundamental difference in manner of origin of the plants belonging to self-and open-pollinated progenies. Such a difference is readily understandable if it be assumed that the plant's own pollen is incapable of fertilizing its own eggs but is capable of initiating pseudogamous development to form apomictic offspring. However, if foreign pollen were available, it is logical to believe that pollen-tube growth would be rapid enough to effect fertilization and produce aberrant forms. An instance of this behavior is strongly suggested by progenies from 1 plant shown in figure 4. Variability in the self-pollinated progeny was 0, the 59 plants conforming perfectly to the parental type. On the other hand, in the open-pollinated progeny only 15 of 59 plants resembled the parent, while the aberrant forms showed the morphological features of triploids. It is possible to select a graded series of behaviors from this extreme case to that of paired progenies which show no differences.

ORIGIN OF OFFSPRING IN POA

Studies on the embryology and on morphological and cytological features of aberrant and matroclinous plants in species of *Poa* have furnished satisfactory evidence of the manner of origin of offspring. In the following classification all theoretically possible methods are outlined:

- I. Reduction division; embryo sac arises by haplospory.
  - A. Nonfertilization.
    - (1) Parthenogenesis; production of haploids.
  - B. Fertilization—
    - (2) By reduced pollen; production of diploids by amphimixis.
    - (3) By unreduced pollen; production of triploids.
- II. No reduction division; embryo sac arises by either diplospory or apospory.
  - A. Nonfertilization.
    - (4) Parthenogenesis: production of diploids by apomixis, with maternal characters and chromosome complement.
  - B. Fertilization—
    - (5) By reduced pollen; production of triploids.
    - (6) By unreduced pollen; production of tetraploids.

Four of the six types have been definitely proved in *Poa pratensis*; namely, types 1, 2, 4, and 5. It has been possible in the present study to determine these types of origin by an analysis of chromosome numbers. Previous work by other investigators leaves little doubt that matroclinous plants in *Poa* arise regularly by type 4 and that aberrant plants arise by type 1, 2, or 5. Recently, Müntzing (*22*) has shown quite conclusively that functional unreduced pollen grains may form in *Poa alpina* L. In this particular case, tetraploids were obtained in the progeny, thus establishing type 6 as an actual method of reproduction in *Poa*. It follows from this observation that type 3 also may possibly be an actual method of reproduction in *Poa*, since there is no reason to assume that unreduced pollen cannot fertilize a reduced egg. The wide range of pollen sizes found in some plants of *Poa pratensis* suggests the possibility that functional unreduced pollen may be formed in this species also.

The appearance of albino seedlings is additional indirect evidence that reduced megaspores may develop into embryos. They may be considered as haploids, since it is unlikely that the high chromosome numbers and autopolyploid relationships found in *Poa pratensis* would permit such frequencies to arise by diploid methods of origin.

The study of polyembryony affords additional evidence of the manner of origin of offspring in *Poa pratensis*. Twin seedlings have been shown to be largely maternal, that is, diploid:diploid, and have probably arisen by the simultaneous development of two nucellar cells. Triploid:diploid twins have arisen in considerable quantities and have been subjected to intensive study. Workers agree that the triploid member has arisen through the fertilization of an aposporous embryo sac by reduced pollen and that the triploid member is invariably weaker and later in starting development. Tinney (*35*) suggested the probable reason for this difference when he reported that one embryo sac nearer the supply of food generally develops at the expense of the weaker member. Andersen (*5*) made the same observation. Haploid:diploid twins have been observed by Müntzing (*20, 21*), Skovsted (*30*), Engelbert (*10*), and the writer, and have presumably arisen by the development of a reduced megaspore and an aposporous egg cell. Of considerable interest is the albino pair of twins observed in the writer's cultures. The most probable constitution of these plants is haploid:haploid, and they must represent the simultaneous development of sister megaspores that have been derived from tke same cell of a dyad after meiosis.

Triple seedlings appearing in the writer's cultures have been shown to be of two types, namely, diploid:diploid:diploid and triploid: diploid:diploid. Triple seedlings have also been reported by Müntzing (*20*). Their origin seems to be no different from that of the corresponding twins. Presumably three instead of two nucellar cells develop.

NATURE AND EXTENT OF VARIABILITY

The average morphological variability (14.8 percent) found by the writer in progenies of Kentucky bluegrass is higher than that reported by other investigators. Åkerberg (*4*) gives 5.9 to 13.4 percent, depending on the nature of his material, whereas Tinney and Aamodt (*36*) give the low value of 1.59 percent. The slight deviations between Akerberg's values and the values obtained by the present writer may well be explained by sampling within a highly polymorphic species. On the other hand, the discrepancies in the writer's reports of variability and those of Tinney and Aamodt need to be accounted for, particularly since the parental material came in part from similar sources.

The writer believes that the most plausible explanation comes from the contrast in the methods employed in germinating the seed for the establishment of the respective nurseries. Tinney and Aamodt germinated seed in soil; the writer utilized Petri dishes for this purpose, thus permitting equal chances for survival of all the products of germination. Since many aberrant plants in progenies of Kentucky bluegrass are weak types barely able to survive, it is readily apparent that this type of plant stands little chance of ever appearing if it must force its way through soil and grow in competition with more vigorous seedlings.

The high correlation between survival in self-pollinated progenies and survival in open-pollinated progenies can mean only that loss of population is not distributed at random throughout the field but is in some way related to an inherent characteristic of the parent. It is logical to relate this loss of plants to the fact that they were forms too weak to survive. If they were too weak to survive, they would not have the genic constitution of the parent and hence should be classified as aberrant. By the proposed criteria for apomixis they must be presumed to have arisen sexually.

The high positive correlation between loss of plants and morphological variation in plants that survive makes the relation between loss and sexuality even more apparent. One cannot escape the conclusion that the two groups of plants should actually be considered as one and that the only difference between them is one of degree of vigor.

These results permit one to suggest that the values for sexuality in Kentucky bluegrass parental plants may be higher than indicated by morphological variation in their progenies, since plants that would have been aberrant never appeared for study. By combining the number of plants that failed to survive and the number of the remaining plants showing morphological variations, we obtain the following percentages: Mean, 25.5±1.5; lowest, 5.0; highest, 90.5; standard deviation, 15.5. The plant and its progeny that gave the highest values are shown in figure 11: Loss, 72.5 percent; variability in remaining progeny, 65.5 percent.

In connection with this treatment of loss of plants and variability, the question may be asked: Why be concerned about plants that are lost even under the most favorable cultural conditions, since they will never appear and therefore will never influence the character of the plant's progeny? In reply, it may be said that this study was designed to determine the extent of apomixis and sexuality in selected plants of Kentucky bluegrass, and no valid criterion of sexuality can be disregarded. Furthermore, by the proper breeding techniques these highly sexual plants may be utilized for hybridization, and therefore any criterion of value in their identification should not be ignored.

It has not been possible in these studies to establish with any degree of certainty correlations between apomixis, as measured by the morphological features of a plant's progeny, and the following measurements that are more easily and quickly determined.

(1) No correlation was observed between apomictic reproduction and source of material. Variability, however, is low in many progenies from plants grown from commercial seed. In fact, the most apomictic types were isolated from commercial seed. This observation is in agreement with the general opinion that apomictic types are more prolific seeders and are thus better fitted to survive under conditions of natural selection. Highly variable forms, however, were obtained from this material, and the mean differences between plants from commercial sources, pasture sources, and strains are not statistically significant.

(2) In regard to correlation between seed set under bag and variability, it was found that in general plants completely sterile under bag gave open-pollinated progenies more variable than did fertile plants. However, exceptions make this relationship difficult to apply to practical work. It is evident that good seed set is no criterion for apomictic reproduction, as some of the most variable progenies came from plants with good seed production under bag. (See fig. 11.)

(3) The simple correlation between germination and variability has a significant negative value, and one might infer that the apomictic types have a higher rate of germination. This correlation, however, is misleading, since the partial correlation drops to an insignificant value if percentage survival of plants in the field is held constant. It seems likely, therefore, that sexually produced embryos are not significantly less viable than apomictically produced embryos in their ability to germinate and that no practical significance may be ascribed to percentage germination as a measure of apomictic reproduction. Therefore the significance of the simple correlation must be caused by the widespread elimination of weak seedlings that would ordinarily go unmeasured in reckoning variability.

(4) Embryological studies by Åkerberg (*4*) and Tinney (*35*) have

indicated that polyembryony and apomictic development should be closely associated phenomena. Therefore, one might reasonably expect a significant negative association between polyembryony and variability. Åkerberg first suggested this relationship and presented rather inconclusive data in its support. The families studied were merely classified as "sexual" and "apomictic," and no statistical treatments or further descriptions were presented. The writer has found a correlation value of -0.205 between variability and polyembryony, which is barely significant ($P=0.05$ at $r=0.180$). It is difficult to infer from this value just what the relationship between polyembryony and apomixis might be. A study of figure 9 suggests the probability of the occurrence of two fundamentally different groups of plants: (1) Those in which there is a high negative association between polyembryony and variability and (2) those in which there is little, if any, association. Granting the probable existence of these two classes, it would seem that in Kentucky bluegrass there are two inherently different types: (1) "Obligate apomicts," in which fertilization rarely occurs and the rate of polyembryony might be a reasonably sound measure of apomictic seed development, and (2) "facultative apomicts," in which fertilization may be readily effected under the proper conditions and in which the rate of polyembryony bears no inverse relation to sexual seed development.

The practical use of the rate of polyembryony as a measure of apomictic seed formation must, therefore, be held in abeyance until greater reliability can be demonstrated for this criterion.

To summarize, correlation studies in Kentucky bluegrass between variability and source, seed set, germination, and polyembryony, respectively, have indicated no statistically significant relationships. Therefore, investigators have no reliable criterion, except the progeny test, for apomictic seed formation in Kentucky bluegrass, and are fully justified in adopting this test as a reliable measure of apomictic and sexual seed formation in parental plants. It should be pointed out, however, that low values for variability will be obtained if cultural practices are employed that surpress the weaker plants.

CONCLUSIONS

The results presented in this report allow one to make the broad generalization that *Poa pratensis* is predominantly an apomictic species. There is, however, a range of apomictic behavior from complete uniformity in progenies to over 70 percent aberrancy, indicating, first of all, the genic nature of the reproductive phenomena, and secondly, the caution that must be used in applying any criterion to a species as highly polymorphic as *P. pratensis*. The writer is strongly of the opinion that we know too little about the species to generalize on any one criterion, whether it be embryology, polyembryony, polyploidy, breeding behavior, or morphological and taxonomic relationships.

PRACTICAL CONSIDERATIONS

The investigations reported in this paper were started with the ultimate purpose of applying the results of fundamental work in *Poa pratensis* to problems of practical breeding. Kentucky bluegrass is important both as a forage and as a turf grass. Improvements are sought in (1) disease and drought resistance; (2) productivity, especially in the period between the spring flush and the secondary fall flush; (3) nutritive value, especially in the fall flush; (4) adaptability to soil and climate factors; (5) association with other species, particularly legumes; (6) tolerance to close grazing and clipping; and (7) palatability.

The progeny tests conducted with 115 parental plants of Kentucky bluegrass indicate that reproduction in this species varies from nearly completely apomictic to highly sexual processes. Theoretically, the identification of such biotypes makes possible the breeding of *Poa pratensis* either as an apomictic or as a sexual cross-pollinated plant.

Available breeding procedures in the improvement of Kentucky bluegrass may be enumerated briefly as follows: (1) Selection of apomictic biotypes that show desirable characteristics, the most rapid and the simplest method of improvement and one in which problems of isolation and maintenance of purity are minimized; (2) utilization of aberrant (sexually produced) plants as material for new apomictic biotypes or for inbreeding and hybridization, depending on their predominant method of reproduction; (3) intraspecific hybridization and strain building, utilizing either sexual biotypes or "facultative" apomicts, in which fertilization is largely effected if foreign pollen is applied; and (4) interspecific hybridization.

Artifically produced species hybrids of *Poa* are, at present, *P. arachnifera* × *pratensis* (Oliver, and later E. Marion Brown, cited by Vinall and Hein (*37, p. 1060*)); *P. pratensis* × *alpina* (Åkerberg (*2*) and Müntzing (*22*)); and *P. compressa* × *pratensis* (Brittingham (*7*)); the first- and last-mentioned crosses resulted in hybrid plants showing agronomic promise.

The experimental evidence presented in this paper does not exclude the possibility that any of the above-mentioned breeding techniques may be applied to the improvement of Kentucky bluegrass.

SUMMARY

From a nursery of 10,000 spaced plants of Kentucky bluegrass grown from seed collected from pasture, commercial, and numbered-strain sources, 115 parental plants, representing a wide range of morphological and physiological types, were selected. An experimental nursery of 10,066 plants was established from seed produced by open-pollination and from seed, where available, produced under parchment bag. Both self-pollinated and open-pollinated progenies from 87 selected plants were available.

Studies conducted in the greenhouse and in the field on sterility and fertility under conditions of bagging showed that about 35.0 percent of the plants regularly had less than 30 percent seed set under bag. Behaviors under bag varied from complete sterility to well over 60 percent seed set. While the physiological effect of the bag may be a factor in seed set, the results indicate that sterility and fertility in *Poa pratensis* are largely genic in nature.

There are significant positive correlations between self-pollinated progenies and open-pollinated progenies in germination, polyembryony, survival in the field, and variability, indicating an inherent nature of the parental plant that produces in the progeny characteristic frequencies of these measurements regardless of the type of pollination. Evidence is presented that variability may be significantly higher in open-pollinated progenies than in self-pollinated progenies of some of the parental plants.

For the offspring of all 115 plants the following average values were obtained: Germination, 80.3 percent; polyembryony, 7.0 percent; survival, 86.3 percent; and variability, 14.8 percent. The highest value obtained for polyembryony was 31.6 percent; the highest for variability, 65.5 percent. The lowest value for survival was 27.5 percent, and this was found in the progeny giving the highest value for variability among the plants that remained.

Variability among the plants from twin seedlings was 16.9 percent and was significantly higher than the value found in plants from seed with a single embryo. However, the same morphological and chromosomal aberrant plants appeared in each population. A study of morphological features, pollen-grain sizes, and chromosome counts has indicated that the aberrant plants from both twin-seedling and single-seedling sources arose from (1) apomictic development of reduced eggs (haploids); (2) fertilization of reduced eggs by reduced pollen (diploids by amphimixis); and (3) fertilization of unreduced eggs by reduced pollen (triploids). The matroclinous plants are thought to have arisen from the apomictic development of an unreduced egg derived from a cell of the nucellus by apospory (diploid by apomixis).

No significant correlations were found between variability and source of material, seed set under bag, and germination, respectively. A barely significant negative correlation existed between polyembryony and variability. The data suggest that in some plants of Kentucky bluegrass a high negative association between polyembryony and variability may be found, whereas in others no association exists. A highly significant negative correlation was found between survival and variability, indicating that variability was generally higher in those progenies that had lost the greater number of plants. This suggests that the plants lost were weak aberrant forms and should be considered in any measure of apomictic seed development in parental plants.

The results indicate that, although *Poa pratensis* may be considered a predominantly apomictic species, such extremes of type and behavior are found that caution is necessary in generalizing on too few data. However, the progeny test appears to be the only practical means of determining the type of seed development of Kentucky bluegrass.

The breeding methods available for improvement of plant types in Kentucky bluegrass are discussed. Selection of desirable apomictic biotypes provides the quickest and easiest method of improvement. If this reaches a limit of usefulness because of the lack of naturally occurring desirable biotypes, the breeder has at his disposal the methods of inbreeding, intraspecific hybridization, strain building, and interspecific hybridization. The results of the study presented in this paper are not inconsistent with the conclusion that any of these methods are applicable to breeding problems in *Poa pratensis*.

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CLEISTOGAMY AND THE DEVELOPMENT OF THE EMBRYO SAC IN LESPEDEZA STIPULACEA [1]

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INTRODUCTION

Lespedeza stipulacea Maxim., commonly known as Korean lespedeza, has become important as a hay, pasture, and soil-conserving crop (*4*).[3] Although numerous investigations have been made on the culture and utilization of this annual, no detailed studies concerning the morphology and development of the flower have been reported. Cleistogamous flowers were first noted in *Lespedeza* in 1840 (*9*) and invariably have been designated as apetalous in contrast to the petaliferous flowers of this genus. McKee and Hyland (*6*) have observed that the occurrence of petaliferous and apetalous flowers is common to most if not all species of the *Eulespedeza* and *Microlespedeza* sections. However, little has been known concerning the conditions that are associated with the development of each flower type. Successful cross-pollinations, varietal or specific, have not been reported in either the annual or the perennial species. More complete knowledge of floral structure and development is desirable before proceeding with a breeding program. The studies on *L. stipulacea* herein reported have been made to determine (1) the morphology of the two types of flowers, (2) the development of the cells of the embryo sac, and (3) the effects of some environmental factors on flower formation.

METHODS

HISTOLOGICAL TECHNIQUE

Some of the material for the histological study was collected from plants growing in the field during 1941, the rest from plants grown in the greenhouse during the fall and winter of 1941 42. The best fixation was obtained by dipping the buds momentarily in Carnoy's fluid and then transferring them to a modification of Bouin's killing fluid, designated as Allen-Bouin II. Good fixation was obtained when the buds were left in the latter fluid 3 weeks. The material was dehydrated in butyl alcohol and infiltrated and embedded in paraffin according to the butyl alcohol method described by Johansen (*5*). Longitudinal sections were cut 8μ to 10μ thick and mounted serially. A combination of safranine and fast green gave the best staining results. Heidenhain's iron-alum haematoxylin stain, counterstained with orange G, was also used with some success.

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[3] Italic numbers in parentheses refer to Literature Cited, p. 272.

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PROCEDURE FOR DETERMINING THE EFFECT OF TEMPERATURE ON FLOWERING

Plants of three strains[4] of *Lespedeza stipulacea* were grown to maturity in the thermo-regulated growth chambers described by Brown (*2*). Observations were made on the kinds of flowers that were formed at different temperatures during the summer under natural conditions of light and during the winter when supplementary lights were used. During the latter period supplementary illumination was supplied, at first by 250-watt Mazda lamps and later by double-unit 40-watt daylight fluorescent lamps, to extend the natural photoperiod to 15 hours. When the plants were 10 inches in height, the photoperiod was reduced to 12 hours to induce floral initiation.

OBSERVATIONS

FLORAL MORPHOLOGY

Gross Morphology

The flowers of *Lespedeza stipulacea* are axillary and are formed on compound racemes. In any one axil, apetalous or petaliferous flowers or a combination of both may be found. The older buds are found near the base of the raceme, but as flowering progresses new buds are formed in the old axils; young buds and mature seeds are commonly found in the same axil.

The petaliferous flowers are typical of those of the Leguminosae; their general morphological features are represented in figure 1, *A* to *E*. Subtending the flower and adhering closely to the calyx are 3 bracteoles (*A*). The calyx consists of 5 undiverged sepals, terminated by 5 lobes, into which the corolla is inserted. All members of the irregular corolla are free except the 2 keel petals (*C*), which coalesce slightly along their anterior margins and surround the pistil and the stamens. The diadelphous stamens (*B*) are 10 in number; the undiverged filaments of 9 of them form a tube, while the tenth is separate.

The apetalous flowers are also complete but differ from the petaliferous flowers in external morphological features by a marked reduction of the corolla, stamens, and pistil (fig. 1, *G–K*). These parts are confined within the tightly closed calyx, which does not open until the developing pod forces it apart. The ovary appears normal, but the style is much reduced and bends downward during development (fig. 2, *A*) so that at maturity it assumes a hooked position with the stigmatic surface pressed directly against the anthers (fig. 2, *B*). Young seed pods from the two types of flowers can usually be identified by the old petal and stamen tissue, which is more persistent though less prominent, on the pods from apetalous flowers (fig. 1, *L*). After this tissue has been discarded the tips of the pods from apetalous flowers usually differ from those of petaliferous flowers by being more hooked or curved. Since the apex of the pod represents merely the base of the style, this point of difference is probably significant only as a means of identification. Pods from both types of flowers are single-seeded, flat, roundish, and reticulate. The average length of a mature apetalous flower is approximately 1.5 mm., that of a petaliferous flower 7.5 mm.

[4] F. C. 19604, F. C. 19601, and Del. 2591.

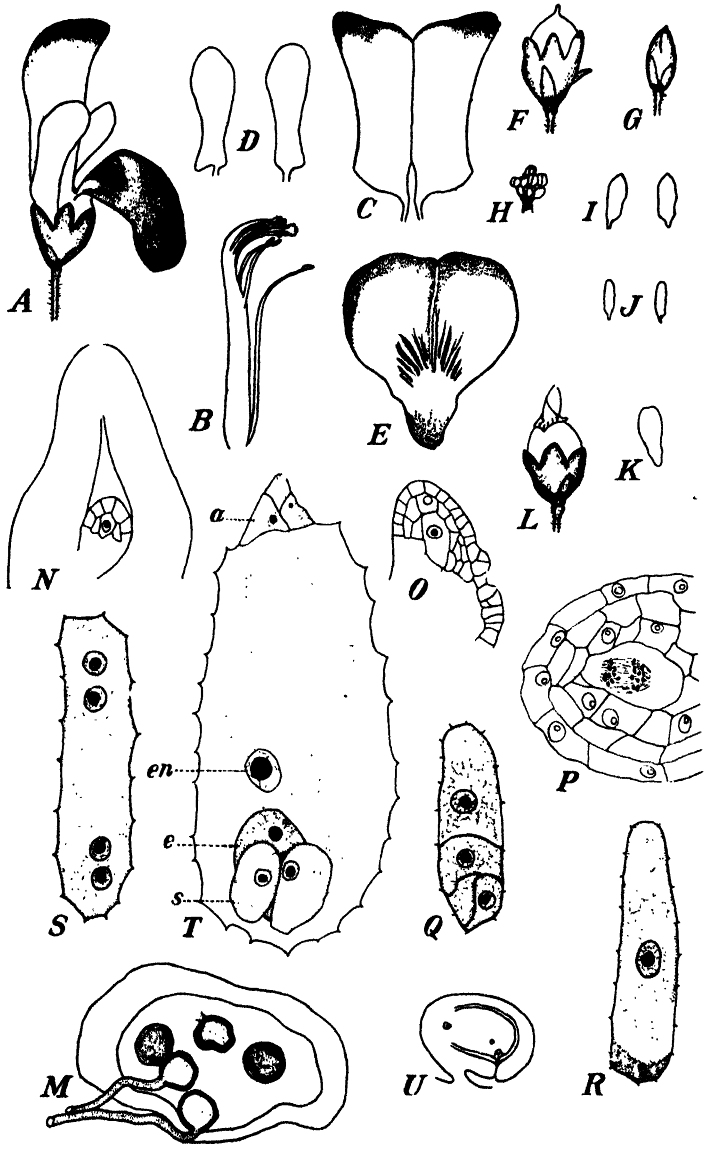

FIGURE 1.—*A–E*, Parts of a typical petaliferous flower, × 5: *A*, Mature flower; *B*, androecium and gynoecium; *C*, keel; *D*, wings; *E*, standard; *F*, immature fruit from petaliferous flower, × 5. G–*K*, Parts of typical apetalous flower, × 5 except *H*, which is × 7½: *G*, Mature flower; *H*, androecium and gynoecium; *I*, keel petals; *J*, wings; *K*, standard. *L*, Immature fruit from apetalous flower, showing remains of corolla, × 5. *M*, Section of anther of apetalous flower, showing penetration of pollen tubes through anther wall, × 325. *N–T*, Megagametogenesis: *N*, Young pistil with ovule, showing archesporial cell, × 325; *O*, older ovule, showing primary parietal and megaspore mother cell, × 325; *P*, midanaphase of heterotypic division; *Q*, megaspores; *R*, surviving megaspore; *S*, four-nucleate embryo sac; *T*, mature megagametophyte of apetalous flower (*a*, antipodal cells; *en*, primary endosperm nucleus; *e*, egg cell; *s*, synergid). *P–T*, × 670. *U*, Formation of campylotropous ovule, × 40.

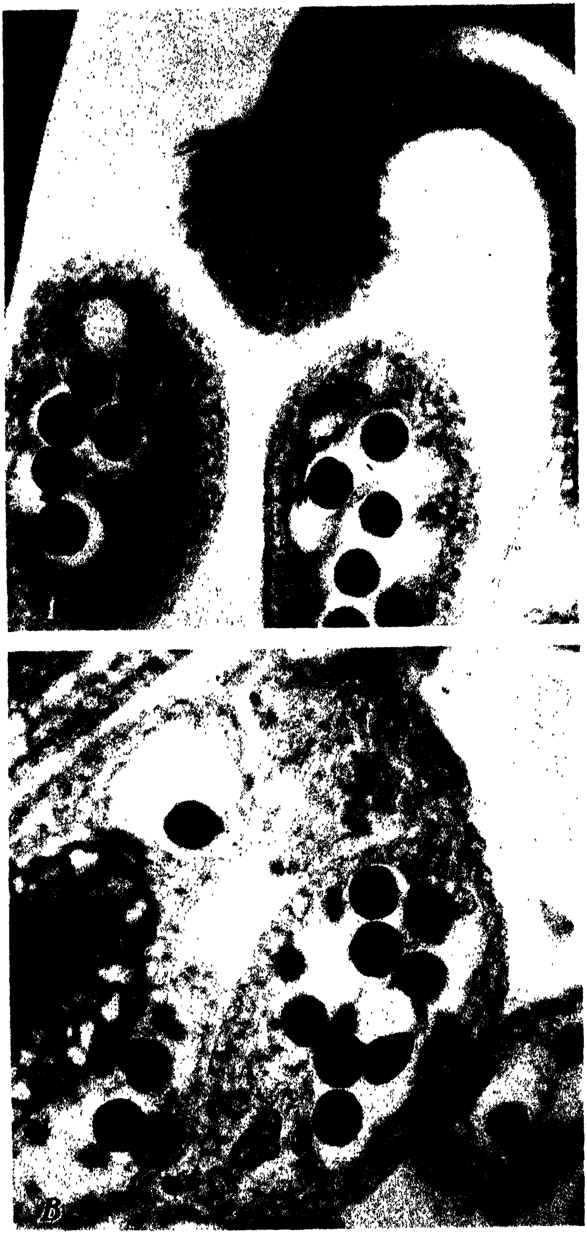

Figure 2.—*A*, Photomicrograph of longitudinal section of immature apetalous flower, showing a portion of the hooked style and the shriveled condition of the pollen grains. × 325. *B*, Photomicrograph of later stage, showing contact of stigma and anthers, and germinating pollen grains. × 375.

An abscission layer is found at the distal end of the pedicel; a slight manipulation of the flower usually results in the severance of the flower at this point.

POLLINATION

The anthers of the petaliferous flowers open during anthesis, shedding the pollen grains against the stigma, which extends slightly beyond the anthers. Generally the stigma is confined within the keel, reducing the possibilities of wind pollination. The pollen grains are well filled and uniform in appearance.

The anthers of the apetalous flowers are indehiscent, the pollen grains germinating within the closed anther sacs (figs. 1, *M*, and 2, *B*). Some of the pollen tubes so formed enter the stigma after penetrating the anther wall. Pollen tubes were observed in anthers touching the stigma as well as in those not in direct contact with this organ. The pollen grains of the apetalous flowers have both a generative and a vegetative nucleus. They are more or less shriveled (fig. 2, *A*), only the better ones germinating. Indications of collapse of the pollen grain wall were observed as early as the time when the megaspore mother cell was being differentiated.

Under normal field conditions approximately 76 percent of the petaliferous flowers formed seeds during the 1941 season. When potted plants of *Lespedeza stipulacea* were grown during the winter months, however, the fertility of the petaliferous flowers was much less than that of the same strains grown during the summer under field conditions. The apetalous flowers are highly fertile during both the summer and winter.

DEVELOPMENT OF THE OVULE

The single ovule develops as a mound of meristematic nucellar tissue arising from the inner surface of the ovary wall near its base. The young ovule is orthotropous (fig. 1, *N*), but as it develops it curves toward the base so that at maturity it is campylotropous (fig. 1, *U*). Shortly after the differentiation of the megaspore mother cell the inner and outer integuments appear as two rounded outgrowths of the epidermis on one side of the ovule (fig. 1, *O*). Shortly thereafter the integuments on the other side develop. When the megagametophyte is mature, both integuments enclose the nucleus except at the micropyle; the inner integuments are two cells thick throughout their entire extent, the outer more than two.

DEVELOPMENT OF THE MEGAGAMETOPHYTE

Soon after the appearance of the ovule an archesporial cell is differentiated in the second layer of cells from the apex (fig. 1, *N*). This slightly enlarged cell has somewhat denser cytoplasm than the surrounding cells and divides to form a parietal and a primary sporogenous cell. The former divides at least once, as is indicated by the fact that the older embryo sacs are usually embedded 2 or more layers within the nucellus. The primary sporogenous cell enlarges, differentiating into a megaspore mother cell (fig. 1, *O*). Figure 1, *P*, represents the migration of 10 chromosomes to each pole of the spindle during the anaphase of the heterotypic division of the megaspore mother cell. The nuclei resulting from this division are increased by the second meiotic division to 4 megaspores (fig. 1, *Q*). The observed haploid chromosome number of 10 is consistent with the somatic number reported by Cooper (*3*). The chalazal megaspore is functional, whereas the other 3 megaspores rapidly degenerate (fig. 1, *R*). The newly formed nuclei of the first division of the surviving megaspore migrate to opposite ends of the embryo sac, where, by 2 more divisions, the usual 8-nucleate condition is formed. Three of the nuclei at the chalazal end develop cell walls, becoming the antipodal cells, which soon degenerate. The fourth nucleus migrates toward the center of the sac to a position not far from the egg apparatus, where it is joined by a nucleus from the micropylar end. They fuse to form the primary endosperm nucleus before fertilization occurs.

The three remaining nuclei at the micropylar end develop cell walls and differentiate into the synergids and an egg cell. Evidence of degeneration in the synergids occurs soon after the fusion of the polar nuclei. The mature megagametophyte of an apetalous flower consists of three degenerate antipodal cells embedded in the nucellus; two synergids, which have begun to degenerate; an enlarged pear-shaped egg; and a large primary endosperm nucleus (fig. 1, *T*).

The general appearance of the mature ovules of petaliferous flowers indicates that fertilization occurs at a later stage than in the apetalous flowers. The nuclei of the synergids are no longer visible and the antipodal cells have so degenerated that they are visible only as darkly stainable material. The entire ovule is larger and the embryo sac has farther invaded the nucellar tissue.

FACTORS AFFECTING FLOWER FORMATION

Observations made on three strains of *Lespedeza stipulacea* grown in thermo-regulated growth chambers showed that both the ratio of petaliferous to apetalous flowers and the total number of flowers formed are conditioned by temperature (table 1). Petaliferous flowers predominated at 80° F. in the summer experiments and at 82° and 95° in the winter experiments; apetalous flowers predominated at 70° in both the summer and winter experiments. At 60° flowering was limited to a few apetalous flowers. At the more favorable temperatures the total number of flowers per plant was too large to count; furthermore, the relative numbers of the two types varied so greatly on the different branches of the same plant that fractional sampling would not have been more accurate than general observations.

TABLE 1.—*Production of petaliferous and apetalous flowers in Lespedeza stipulacea at different temperatures*

Period	Temperature °F.	Petaliferous flowers	Apetalous flowers
May 15—Sept. 15, 1941	80	Abundant	Few.
	70	None to few	Abundant.
	60	None	Very few.
Oct. 29, 1941—Feb. 7, 1942	95	Abundant	Few.
	82	do	Few to many.
	70	Very few	Abundant.

It has been observed throughout these experiments that temperature is not the only factor involved in the determination of flower type. The amount of development that the plant has undergone when the reproductive processes are initiated affects the proportion of each flower type. For instance, plants grown without the aid of supplementary light during the period of the winter when the days were shortest became reproductive when they were only about 2 inches in height and produced few if any petaliferous flowers at temperatures of about 80° F. When plants grown during the same period were first exposed to a 15-hour photoperiod until they had grown to a height of 10 inches and to a 12-hour photoperiod thereafter, numerous petaliferous flowers were formed.

Light intensity and length of day probably are factors also affecting the relative numbers of petaliferous and apetalous flowers. Cloudy weather seems to retard the formation of petaliferous flowers. It is commonly known that apetalous flowers are more numerous during the winter months, even though artificial lights are used.

Finally, individual potted plants frequently differ from one another in respect to flower formation when grown under controlled temperatures and identical light conditions. It is believed that the nutrition of the plant may be so altered by conditions in the soil as to have a marked effect on the ratio of the two flower types formed.

DISCUSSION

An unusual method of pollination has been described for the apetalous flowers of *Lespedeza stipulacea*. Madge (*7*) and others have reported similar mechanisms of pollination in the cleistogamous flowers of *Viola*. Parks (*8*) found that the anthers of the closed flowers of *Commelinantia pringlei* (S. Wats.) Tharp dehisce but that most of the pollen remains in the anther cavity.

One of the significant results of this investigation was the demonstration that the proportion of petaliferous and apetalous flowers is conditioned largely if not entirely by environmental conditions. When one or more of the factors become unfavorable for the formation of petaliferous flowers there is a reduction in the number of flowers of this type. Response to such a change is not always definite; gradations exist from plants producing only apetalous flowers to those that are highly petaliferous. McKee and Hyland (*6*) found that *Lespedeza cuneata* (Dum. de Cours.) G. Don formed 75 percent of its seed from petaliferous flowers in 1939 and only 31 percent in 1940. Differences in light intensity and day length during the flowering seasons were considered possible factors in this variation. Some of the species investigated by these workers produced more seeds from petaliferous flowers; others formed the greater proportion of their seeds from apetalous flowers.

Some of the factors that condition flowering in *Lespedeza stipulacea* have been found to affect flowering in other groups of plants where cleistogamy occurs. Bergdolt (*1*) found a decrease of cleistogamous flowers with increased soil fertility in *Viola*. Vöchting (*10*) reported that with some plants, such as *Stellaria media* (L.) Cyrill. and *Lamium purpureum* L., chasmogamous flowers were formed under ordinary light conditions, whereas under feeble light the flowers remained closed with the conspicuous flower parts imperfectly developed. Parks (8) reported that cleistogamous flowers of *Commelinantia pringlei* would open if exposed to light. The association of reduced light with the appearance of cleistogamous flowers is consistent with the writer's observations in *Lespedeza.*

SUMMARY

Lespedeza stipulacea Maxim. bears two kinds of flowers, the apetalous and the petaliferous.

The apetalous flowers are highly fertile; the fertility of the petaliferous flowers is variable, depending on the conditions under which they were formed.

The pollen of the apetalous flowers germinates in the unopened anther sacs, and owing to the proximity of the stigma to the anthers, some of the pollen tubes enter the stigma after penetrating the anther wall.

By two meiotic divisions the megaspore mother cell forms a row of four megaspores. The chalazal megaspore gives rise to an eight-nucleate, seven-celled embryo sac. The two polar nuclei fuse before fertilization takes place.

The proportion of apetalous and petaliferous flowers is determined largely, if not entirely, by environmental factors. Certainly temperature is a factor, since flowering is predominantly petaliferous at 80°, F. and apetalous at 70°. Other factors, such as the initial development of the plant at the time of floral initiation, light intensity, day length, and soil conditions, are probably involved also.

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INFLUENCE OF TEMPERATURE, MOISTURE, AND SOIL REACTION ON THE DAMPING-OFF OF RED PINE SEEDLINGS BY PYTHIUM AND RHIZOCTONIA[1]

By L. F. Roth, formerly *research assistant*, and A. J. Riker, *professor, Department of Plant Pathology, Wisconsin Agricultural Experiment Station*[2]

INTRODUCTION

The influence of environment on damping-off has been studied because of the great variability in the occurrence of the disease both in the same nursery from season to season and in different nurseries during the same season. There also may be great variation in different parts of the same nursery during the same season. A number of instances of this variability are recorded in the reports dealing with the fungi responsible.

The two primary causal agents in Wisconsin are *Pythium* and *Rhizoctonia* (*16*).[3] The literature relating to the activity of these organisms as they attack forest seedlings and various other plants is extensive. Much of that pertinent to this topic has been reviewed by Hartley (*7*), C. Roth (*15*), L. F. Roth,[4] and Ten Houten (*19*). The fungi causing damping-off of particular hosts may vary strikingly, occasionally within the same species, in their responses to environmental factors. This variability is doubtless influenced in turn by the usual increase of resistance by the seedlings with increase in age.

The purpose of the present study was to determine the influence of temperature, moisture, and soil reaction, respectively, on the damping-off of red pine (*Pinus resinosa* Ait.) in Wisconsin in regard to (1) germination of the seed and growth of the host, (2) vegetative growth of the two pathogens named, and (3) the interrelation of host and parasite for the development of disease.

MATERIALS AND METHODS

The pathogenic fungi used most were cultures F-111-A of *Pythium irregulare* Buisman and F-5 of *Rhizoctonia solani* Kühn. Descriptions of these organisms are given elsewhere (*16*). In addition, laboratory studies were made with a culture of *Pythium debaryanum* (F-117) kindly supplied by W. F. Buchholtz and a particularly virulent strain of *Rhizoctonia* (F-118) isolated from seedlings in central Wisconsin.

The red pine seed used was from local stock collected recently and stored in tightly sealed bottles at about 40° F.

The soil was Plainfield sand secured from a 20-year-old stand of jack pine adjacent to the Griffith State Nursery at Wisconsin Rapids.

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[2] Acknowledgment is made to the Wisconsin Conservation Department for its cooperation and encouragement in these investigations. Assistance in many of the tests was furnished by the personnel of the Work Projects Administration, Official Project No. 65-1-53-2349. The writers are indebted to Eugene Herrling for assistance in preparing the illustrations.

[3] Italic numbers in parentheses refer to Literature Cited, p. 284.

[4] Roth, L. F. THE INFLUENCE OF ENVIRONMENTAL FACTORS UPON THE DAMPING-OFF DISEASE OF CONIFER SEEDLINGS CAUSED BY PYTHIUM AND RHIZOCTONIA. 1940. [Unpublished doctor's thesis. Copy on file, University of Wisconsin library, Madison, Wis.]

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This soil was used without further sterilization because it was practically or entirely free from *Pythium* and *Rhizoctonia*. Its reaction was pH 5.5. An analysis provided by S. A. Wilde showed the following approximate composition: Silt and clay, 10.5 percent; base exchange, 5.5 milliequivalents per 100 gm.; total nitrogen, 0.1 percent; organic matter, 3.7 percent; available phosphate, 35 pounds per acre; available potash, 150 pounds per acre; and replaceable calcium, 2.9 milliequivalents per 100 gm.

The soil showed no tendency to puddle or cake under varying moisture conditions.

Germination was hastened from about 4 days to a month, according to the temperature, by keeping the seed 4 days on moist filter paper at 28° C. This treatment saved much time and had no apparent ill effects on the seedlings. The period between germination and emergence remained unchanged.

The more common methods for inoculating soil were found less satisfactory than the following: About 100 clean red pine seedlings, from 1 to 3 weeks old, were cut at the ground level and chopped into pieces 1 to 5 mm. long. In each of the 90-mm. Petri dishes used in growing the inoculum, 50 to 100 of these pieces of fresh tissue were mixed with water agar just before solidification. The plates were inoculated at the center and incubated at laboratory temperature. The damping-off fungi covered the agar before any contaminants introduced on the chopped seedlings became established. The agar mycelial mat was cut into 2-mm. squares. One Petri dish served for one 6-inch round container in which 100 pine seeds were planted. Smaller containers received proportionately less inoculum and seed. The inoculum was distributed uniformly over the soil surface, the seed was planted at the same level, and the whole was covered with about three-sixteenths inch of soil.

The amount of disease was based upon the proportion of individual seedlings destroyed. After the seed came up, counts were made every 3 days for about 4 weeks, by which time the seedlings had developed resistance. After the final count, records were prepared of total postemergence damping-off, survival, and total emergence. Parallel, uninoculated controls were always provided. The percentage of emergence was calculated from that in the controls. The preemergence damping-off was calculated by subtracting the percentage of total emergence in the inoculated soil from that of emergence in the controls.

The identity of the fungi causing the disease was determined in all cases. Representative diseased seedlings up to a dozen, selected according to their suitability for plating, were culturally examined on water agar. In every case only the culture introduced was recovered.

Measurements of host development as determined by height of above-ground parts and weight of entire seedlings, wet and dry, were inadequate. Root length alone varied uniformly in Plainfield sand in relation to temperature and acidity. Consequently, the mean root length of 50 seedlings taken at random and washed out of the control containers has been used as the measure of host development.

TEMPERATURE

TEMPERATURE AND THE PATHOGENS IN CULTURE

The influence of temperature upon growth of two isolates of *Pythium* and two of *Rhizoctonia* was examined. For each isolate four Petri dishes of potato-dextrose agar, pH 5.8, were seeded in the center with a 2-mm. culture-bearing disk of similar agar. Four dishes were wrapped in wax paper and incubated at each of nine temperatures, ranging in 4° intervals from 4° to 36° C. Of measurements made at 12-hour intervals after inoculation, those made at 36 hours showed the most satisfactory range and were best suited to graphic presentation. The mean values for the four colony diameters at each temperature in one of three separate trials are shown in figure 1.

Growth of the four strains was best at 28° C. and good between 18° to 34°. Throughout this range up to about 34° *Pythium* grew more rapidly than *Rhizoctonia*. From 12° to 32° growth of the 2 strains of *Rhizoctonia* was similar, and that for the two species of *Pythium* was similar. However, at 36° one strain of *Rhizoctonia* showed little decline while the other and the two of *Pythium* were declining rapidly. Although the good vegetative growth of both groups between 18° and 32° suggested a preference for warm temperatures, the effective pathogenicity of *Pythium* extended well down into the cooler temperatures. While growth was slower, much more time was available at cooler temperatures because of the reduced growth rate of the seedlings and their consequently prolonged susceptibility.

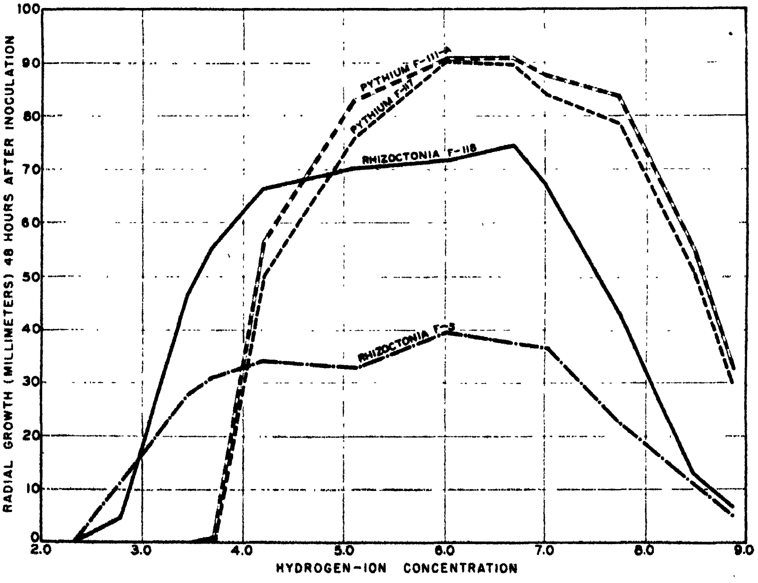

FIGURE 1.—Radial growth in millimeters of four damping-off fungi on potato-dextrose agar incubated at various maintained temperatures.

TEMPERATURE AND THE GERMINATION OF RED PINE SEED

The germination of red pine seed was examined in the larger experiments dealing with pathogenicity. In this case the controls, which ran parallel to seed in inoculated soil, were used. The tests were made in Wisconsin soil-temperature tanks (*11*) with temperatures ranging from 12° to 33° C. at 3° intervals. Two tanks were placed in each of 4 constant-temperature houses, thus: 12° and 15° tanks were in a 16° house, 18° and 21° tanks were in a 20° house, 24° and 27° tanks were in a 24° house, and 30° and 33° tanks were in a 28° house.

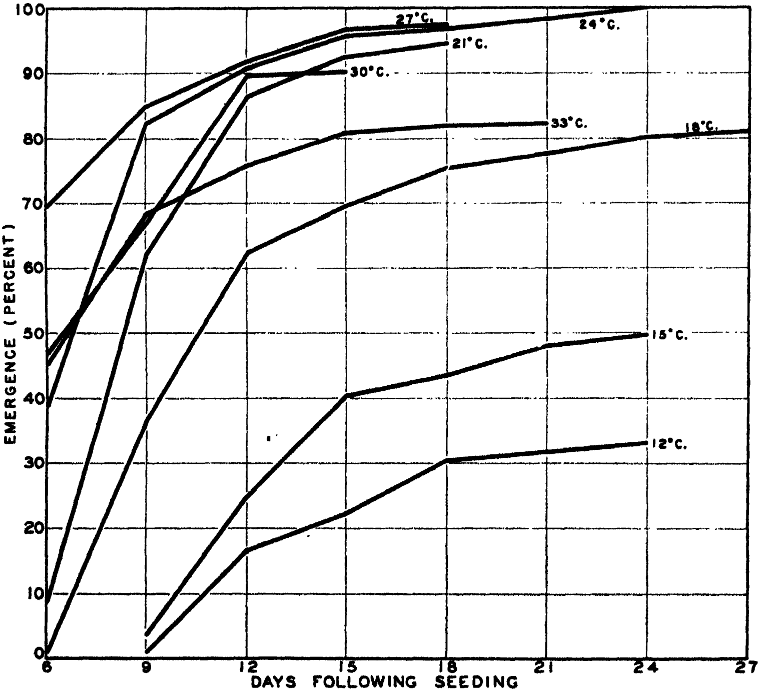

FIGURE 2.—Rate and amount of emergence of red pine seed at different soil temperatures (C.).

Each tank accommodated 8 cylindrical cans 6 inches in diameter and 6 inches deep. Out of every 8 cans, 3 were inoculated with *Pythium* and 3 with *Rhizoctonia*, while 2 were uninoculated controls. The cans were placed in the tanks in a random manner. Germination was uniform, except at 12°, where the variation was not significant.

Throughout these temperature studies the soil was commonly maintained at 60 percent of its moisture-holding capacity against free drainage by frequent weighing and by adding water uniformly to the surface when necessary to restore the original weight. The customary central watering tubes served primarily as supports for covers of nonmoistureproof cellophane. These covers reduced surface evaporation, by their nonmoistureproof character prevented complicating drip from water of condensation, and eliminated contamination between cans.

The temperature strongly influenced both the rate and the amount of emergence. The best emergence was secured at 24° C., where 89 percent of seed planted came up. For easy comparison of the effects of temperature, the best emergence is considered as 100 percent and the percentage emergence at other temperatures determined accordingly. The results of four different experiments have been averaged for the curves in figure 2. Emergence was poor at 12° and 15°, good at 18° and 33°, and excellent from 21° to 30°.

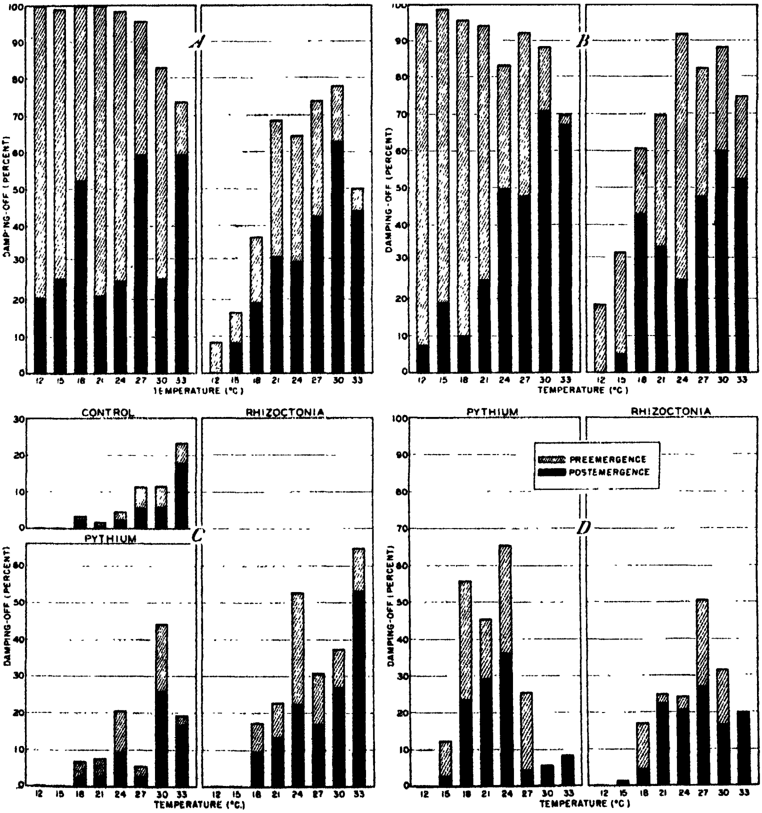

FIGURE 3.—Total preemergence and postemergence damping-off of red pine seedlings in Plainfield sand caused by *Pythium* and *Rhizoctonia* at different temperatures: *A*, Steamed, moisture at 60 percent of moisture-holding capacity, pH 6.4; *B*, same as *A* except for pH, 4.8; *C*, unheated forest soil, moisture at 60 percent of moisture-holding capacity, pH 5.5; *D*, steamed, moisture at 40 percent of moisture-holding capacity, pH 5.5.

TEMPERATURE AND DAMPING-OFF

Warm temperatures are generally considered (*10*, *7*) to favor either the amount of damping-off or its rate of appearance on conifers. C. Roth (*15*) found damping-off fungi most destructive at intermediate temperatures while Gravatt (*5*) observed that *Rhizoctonia* was most aggressive at low temperatures. Such differences are not surprising in view of the variability of the different fungi and the different hosts involved. Similarly, other varying factors of the environment influence the temperature response. On hosts other than forest seedlings various workers have reported great variation in the temperature relations of *Pythium* and *Rhizoctonia* (as reviewed by L. F. Roth (1940) [5]).

The effect of controlled temperature on damping-off by both *Pythium* and *Rhizoctonia* was examined in 11 series of soil-temperature studies between 1937 and 1940. When free from biological competition, *Pythium* commonly killed most of the seedlings at 12° C. and only about half of them at 33°, with intermediate results between these temperatures. *Rhizoctonia* started with relatively few damped-off at 12° but approached a maximum of between 50 and 98 percent at warmer temperatures. This maximum varied with other conditions but was fairly well maintained between 20° and 30°. A sharp decline followed at 33°. Thus both fungi were active at the temperatures favorable for host development, *Pythium* having some advantage at cooler temperatures, *Rhizoctonia* some at warmer temperatures. The details on most of these studies are omitted because of their volume and because the summary on those reported in detail is representative.

The effect of temperature was modified, particularly by soil moisture and reaction. The influence of these factors is graphically summarized in figure 3, which shows the results in four different tank series set up with several varying lots of Plainfield sand. These series have been selected because they represent certain important variations deserving attention. The soil in series *A*, *B*, and *C* held moisture at 60 percent of its moisture-holding capacity and that in series *D*, 40 percent. The soil in *C* and *D* was forest soil secured near the nursery, and that in *A* and *B* was soil from the nursery. *A* had been treated with lime and *B* with sulphur 1 and 3 years earlier, respectively. The reaction of *A* was pH 6.4; *B*, 4.8; *C* and *D*, 5.5. The soil in *A*, *B*, and *D* was autoclaved for 10 minutes at 15 pounds pressure to kill any damping-off fungi that might be present. There was no damping-off in the controls of the heated soil. That in the unheated resembled the damping-off caused by *Fusarium* (C. Roth, *15*).

Total damping-off caused by *Pythium* and *Rhizoctonia* at each of the eight temperatures for the four series is shown by vertical bars in figure 3. The solid part of each bar represents damping-off after emergence and the shaded part, preemergence loss. As explained before, for easy comparison of the effect of damping-off, independent of variations in emergence at different temperatures, the emergence in the controls has been taken as 100 percent. The isolate of *Pythium* was more virulent than that of *Rhizoctonia*. Consequently, where other factors did not influence the relationship, the attack by *Pythium* in these particular experiments was more effective than that by *Rhizoctonia*. In other studies this difference between *Pythium* and *Rhizoctonia* was not apparent.

The amount of seedling growth at different temperatures was recorded. At the close of each of these 4 temperature experiments, the seedlings from the control cans at each temperature were washed free of soil. From each can 25 were selected at random and the root

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[5] See footnote 4, p. 265.

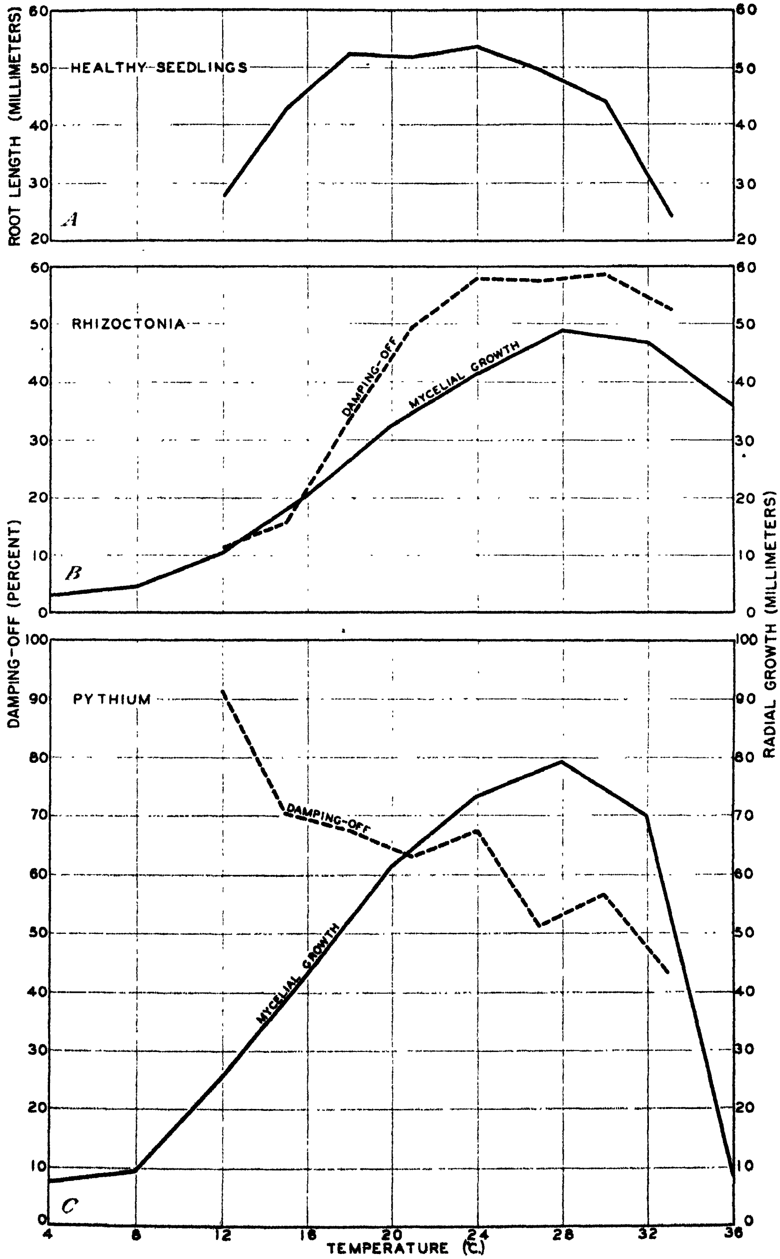

FIGURE 4.—The influence of temperature on: *A*, Root growth of healthy seedlings; *B*, growth of *Rhizoctonia* in culture and the percent of damping-off; *C*, growth of *Pythium* in culture and the percent of damping-off.

lengths measured. Since there were 2 cans in each of the 4 experiments, each point on the curves in figure 4 represents the average of 200 seedlings.

The responses to different temperatures of the vegetative growth by host and parasite and of the damping-off when the two are acting together are summarized in figure 4. The different items are placed in juxtaposition for easy comparison. The growth of the red pine seedlings as indicated by root length is given in *A*. This curve shows that red pine developed well between about 15° and 30° C. At 30° and 33°, the warm temperature induced spindling growth, weak hypocotyls, and pale color, as well as poor root development. Deficient growth appeared also at temperatures below 15°, particularly in soil having a low moisture content. The size of mycelial mats in culture and the percentage of damping-off by *Rhizoctonia* are shown in *B* and those of *Pythium* in *C*. The amount of damping-off is the average from several tank series in which conditions were favorable for the development of damping-off.

Where moisture conditions were favorable and the pathogen was not competing strongly with other fungi (fig. 3, *A* and *B*), total damping-off caused by *Pythium* decreased from a broad maximum lying between 12° and 21° C. to a minimum at 33°. The opposing curves of fungus growth and damping-off (fig. 4, *C*) at cooler temperatures are quite striking.

When *Pythium* was exposed to the competition of the abundant microflora and fauna of the nonautoclaved forest soil (fig. 3, *C*) but was under favorable conditions of soil moisture, the effect of temperature was quite different. No damping-off occurred at either 12° or 15°. Above 15° damping-off increased to a maximum at 30° and then fell off at 33°. Platings of damped-off seedlings from all *Pythium* cans of this series were made. Only at 24° and 30° C. (fig. 3, *C*) did the number of isolates of *Pythium* exceed that of the unidentified fungus resembling *Fusarium* found in the controls. These results emphasized the importance of biological competition as a factor influencing damping-off. A study of this aspect, however, is beyond the scope of the present paper.

Soils of pH 4.8 showed somewhat less postemergence damping-off by *Pythium* at the cooler temperatures than soils with pH 6.4.

At cool temperatures *Pythium* was most easily stimulated by other conditions favorable to aggressive growth but was also most quickly inhibited by unfavorable conditions. Since various competing microorganisms are active in nature and since the moisture of the soil is often low, *Pythium* development in the nursery is likely to be greatest at temperatures between 18° and 24° C.

Greatest *Rhizoctonia* damping-off in all series (fig. 3) occurred at moderately warm temperatures. As temperature became unfavorable for parasite development, damping-off decreased rapidly to a minimum near 12° C. The similarity of curves in figure 4 for fungus growth and damping-off is striking. As was the case with *Pythium*, differences in pH of 4.8 and 6.4 had little if any influence on the trend of total damping-off. The magnitude of the *Rhizoctonia* loss, however, was significantly greater in the more acid soil. Biological or soil-moisture factors in these studies failed to influence *Rhizoctonia* to the degree that they affected *Pythium*. The *Rhizoctonia* attack at 33° in series

C was approximately that in either *A* or *B*, suggesting perhaps that it was inhibited by warm temperature less than its competitors. At cooler temperatures, however, competition seemed to diminish the attack. The height of the postemergence bars in figure 3, *D*, indicates that *Rhizoctonia* damping-off in dry soils did not respond to temperature between 18° and 33° with the variability shown in wet soil. Below 18° in both dry soil and unheated soil, damping-off by both fungi fell off to insignificance.

Considering the four series as a whole, the general trend of postemergence damping-off for both fungi was up with rising temperature. Such a response probably accounts for the established belief that damping-off is most severe at warmer temperatures.

However, in the graphs for *Pythium*, postemergence damping-off appears an inadequate measure of actual injury. At the cooler temperatures in moist soil and in the absence of competition (fig. 3, *A* and *B*), losses through preemergence damping-off appear excessive and unrelated to the rate of vegetative growth of the fungus (fig. 4, *C*). It is apparent, however, that at 12° C., the lowest temperature allowing germination and measurement of damping-off (figs. 2, 4, *C*), the fungus grew rapidly enough (fig. 4, *C*) to allow reasonably prompt dissemination throughout the container. This high loss appears, therefore, to be correlated with slow germination and seedling development (fig. 4, *A*). At cool temperatures in dry soil (fig. 3, *D*) and with competition (fig. 3, *C*) the proportion of preemergence to postemergence damping-off by *Pythium* diminishes to not much above 1 : 1.

With *Rhizoctonia*, however, the proportion of postemergence to preemergence damping-off appears relatively constant at all except warm temperatures and is roughly 1 : 1. A correlation between temperature, preemergence loss, and the vegetative growth of the fungus is evident. At cooler temperatures, slow fungus growth seems a factor limiting disease development. Ramification throughout the soil or extension beyond the point of original infection was so retarded that no rise appears in the damping-off curve even though slow emergence (fig. 2) and development of the seedlings exposed them for a longer time to fungus attack (fig. 4, *A*). With rising temperatures favoring mycelial growth the curve for damping-off also rises (fig. 4, *B*). The decline in *Rhizoctonia* damping-off above 30° C. appears similarly correlated with poor fungus development.

MOISTURE

SOIL MOISTURE

The importance of soil moisture for damping-off is accepted generally, but there is considerable divergence concerning details (*7, 20, 15, 6*). The subject has been reviewed by L. F. Roth.[6] This lack of agreement has doubtless arisen from various conditions other than moisture. To clarify these influences, several experiments have been made testing the effect of varying moisture in Wisconsin nursery soil.

The maintenance of the desired moisture content of the soil, particularly at the all-important top half inch, presented various problems. Preliminary experiments were conducted with 2-quart crocks in which the moisture content was regulated by the weighing method. While the results were in general agreement with those given later, so much variability arose from inadequate moisture control that the details have been omitted.

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[6] See footnote 4, p. 265.

The method used depended on the uniform capillary rise of water in the Plainfield sand. Thirty-six galvanized-iron 4-inch cylinders, each having one end covered with galvanized fly screen, were prepared. The cylinders varied in height, sets of six being 4.5, 6, 7.5, 9, 10.5, and 12 inches, respectively.

Two watertight, flat-bottomed pans 14 by 28 inches with 3-inch rims accommodated the cylinders. Each cylinder, with a sheet of filter paper over its screen bottom, was completely filled with Plainfield sand, pH 5.5. The soil was packed by dropping the cylinders 1 inch 15 times. After the 10.5- and 12-inch cylinders had been dropped twice, they were again filled and dropped the remaining 13 times cylinders of the 2 intermediate heights were dropped once before they were refilled while the 2 shorter groups were dropped 15 times without refilling. The packed soil in all cylinders came within about an inch of the top. The cylinders were arranged in graduated order in the pans in which one-half inch of freely circulating water was maintained. The temperature during these moisture studies fluctuated around the favorable 24° C. After 25 seeds had been planted and the soil in the top of each soil cylinder had been inoculated according to the method described for the temperature studies, a piece of nonmoistureproof cellophane was fixed over each cylinder. A cheesecloth cover was placed over the entire unit to prevent excessive light and evaporation. The relative humidity of the greenhouse air varied around 50 percent. An experiment in progress is shown in figure 5.

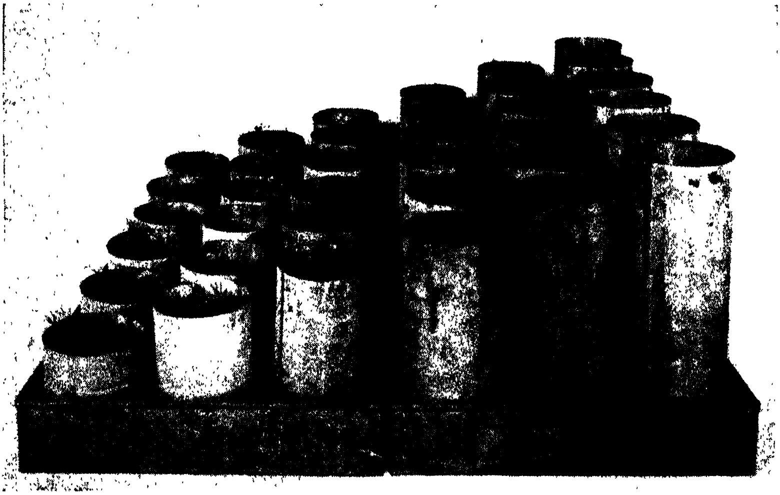

FIGURE 5.—Soil moisture equipment in which the moisture of the surface soil was regulated by the height of the capillary column from the water in the pans. A nonmoistureproof cellophane cover was commonly placed over each cylinder.

The efficiency of this method for controlling the moisture content in the surface layers of the soil used is shown in figure 6, *A*, where the

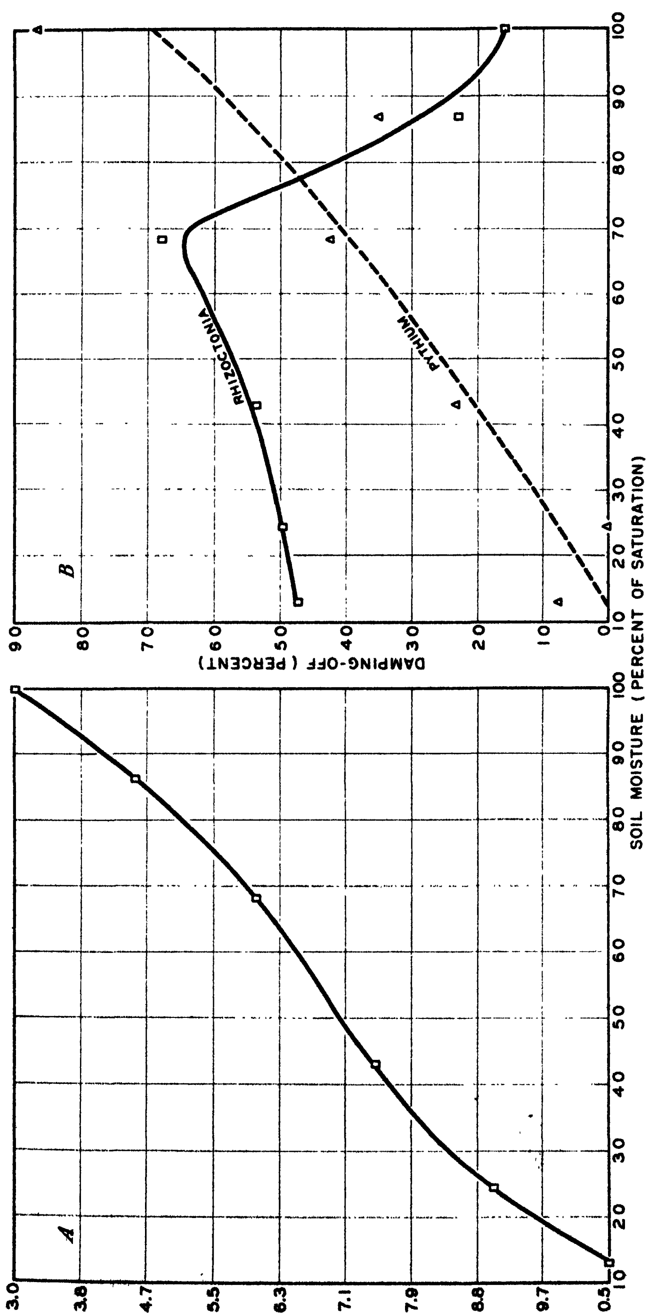

FIGURE 6.—Soil moisture and damping-off: *A*. Moisture content of the upper one-half inch of soil, showing efficiency of soil columns of graduated heights for regulating surface moisture; *B*, damping-off of red pine in virgin Plainfield sand, inoculated with *Pythium* and *Rhizoctonia* under the moisture conditions shown in *A*.

moisture content of the upper one-half inch of soil is plotted against the height of the soil column in inches above the water level. The curve shows a progressive increase in soil moisture, from 13 percent to 100 percent saturation, corresponding with decrease in column height. Since the minimum moisture for germination lies between 30 and 35 percent, it was necessary at the beginning to add some water at the surface of the two tallest series of cylinders. After germination, but before emergence, the moisture level was allowed to assume normal value.

The amount of moisture for host development varied with columns of different height. Average root lengths of seedlings washed from uninoculated control containers in a representative experiment were as follows:

Root length (millimeters)	57, 48, 46, 43, 43, and 38
Soil moisture (percent)	13, 25, 43, 68, 86, and 100

In soil with 13-percent moisture the root elongation was rapid. Growth was straight down with some lateral branching. The roots had a relatively dark color, suggesting early suberization, which, if anything, probably increased their resistance to damping-off. In the saturated soil, root growth was very slow and tortuous, branching was abundant, and the main roots penetrated little below the original depth of the seed. The roots remained succulent and unsuberized for a month and thus probably maintained susceptibility. In the four soils with intermediate moistures, root lengths and appearance were also intermediate.

In each soil moisture series two cylinders of each height were inoculated with *Pythium* and two with *Rhizoctonia*. The remaining two were left uninoculated as controls. Each cylinder was planted with 25 red pine seeds. The experiments were repeated four times. While all the findings were essentially similar, some variability was observed. The results of a representative series are given in figure 6, *B*, where the damping-off, expressed as percentage of emergence in the controls, is plotted against soil moisture. In this case 87 percent of the seedlings damped-off in the *Pythium* inoculations in saturated soil. However, probably because of error, only about 35 percent damping-off appeared in the *Pythium* inoculations at 86 percent moisture. Such a drop at this point was not found in three other experiments.

The curve of *Pythium* damping-off in figure 6, *B*, rises steadily with increasing moisture. The curve for *Rhizoctonia* damping-off rises very gradually as soil moisture increases from 13 to 68 percent of capacity. After this point there is a marked decline of damping-off to a minimum at 100 percent moisture. The entire range studied below about 80 percent may be considered favorable to *Rhizoctonia* damping-off while soil moisture greater than this appears to inhibit the attack, provided atmospheric humidity is relatively low.

AIR HUMIDITY

The importance of the relative humidity of the air appeared in one soil moisture experiment when the nonmoistureproof covers were replaced by moistureproof cellophane placed tightly over the tops of the cylinders. In those having above 70 percent soil moisture, infection increased to 100 percent damping-off at saturation. All cylinders with soil moisture above 60 percent showed abundant aerial mycelium. At high humidities the seedling cotyledons were commonly attacked.

The influence of air humidity as well as soil moisture upon damping-off was studied in view of this response. Seventy-two 4-inch pots were filled with clean, untreated Plainfield sand, pH 5.5, planted with 35 red pine seeds, and divided into 3 equal lots. One lot was inoculated with *Pythium*, one with *Rhizoctonia*, and the third was left uninoculated. Since the soil was not sterilized, a small amount of damping-off occurred in the controls, particularly those at 90 percent moisture. Twelve of each inoculation series were adjusted to approximately 90 percent saturation and the remaining 12 to 35 percent. Six of the pots of each moisture group were placed in a cheesecloth-covered cage having a relative humidity of approximately 50 percent. The other 6 pots were kept in a glass humidity chamber equipped with a spray and a circulation fan. Greenhouse temperatures in both cases were about 24° C. Only postemergence damping-off was considered in interpreting the results. The data are given in table 1 without correction for germination. As in the preceding experiments, soil moisture greatly influenced damping-off. The loss in pots inoculated with *Pythium* and containing 90 percent moisture was 90 percent or greater while less than 1 percent damped-off in the dry pots. Air humidity had little influence on the amount of damping-off in pots inoculated with *Pythium*. However, high air humidity increased the loss caused by *Rhizoctonia*, irrespective of soil moisture. As in the studies on soil moisture, *Rhizoctonia* losses were greater in dryer soils.

TABLE 1.—*Influence of soil moisture and air humidity upon damping-off by Pythium and Rhizoctonia*

Fungus inoculated	Soil moisture[1]	Approximate relative humidity	Emergence	Postemergence damping-off	
	Percent	*Percent*	*Number*[2]	*Number*	*Percent*
Control	90	98	147	20	14
		50	163	19	12
	35	98	182	9	5
		50	156	0	0
Pythium	90	98	140	138	99
		50	108	97	90
	35	98	188	1	0
		50	155	2	1
Rhizoctonia	90	98	94	63	67
		50	91	39	43
	35	98	106	89	84
		50	109	61	56

[1] Approximate percent of moisture-holding capacity. The moisture content was maintained by subirrigation.

[2] 210 seeds were planted in the 6 pots of each treatment.

ACIDITY

The adjustment of soil reaction has been commonly and often successfully used to control damping-off. Thus the influence of acidity of the medium upon growth of the fungi in culture, as well as the effect of soil reaction upon both the host and disease development, becomes of especial interest.

The success of acid treatments in disease control, considered with the observation of Gifford (*3*) that applications of lime and wood ashes increased damping-off, has led to the general conclusion that the disease is favored by neutral or alkaline soils. Wilde (*21*) considers the most desirable reaction for nursery soil to be between pH 5.0 and 6.0. He has stated that "* * * a reaction of soil higher than pH 6.5 is highly undesirable in the nursery since it provides the optimum condition for the development of damping off fungi."

Studies on the distribution in sandy Wisconsin soils of the two fungi considered have shown that *Pythium* predominates (*16*) in soil more alkaline than about pH 5.8 and *Rhizoctonia* in soil more acid. Likewise, in figure 3 it appears that the relative activity of these two fungi was altered by a change in reaction. This relationship was not found on soils of calcareous origin. On heavy soils, even as acid as pH 4.2, *Pythium* was found abundant. This observation is in accord with the opinion of Hartley (*7*) and of Gäumann (*2*) that humus content influences the abundance of *Pythium*.

The above observations suggest that damping-off may not be closely limited to slightly acid or neutral soils but may, in fact, be favored by moderately to highly acid soils. They are in accord with the suggestion that part of the effectiveness of acid treatment comes from partial soil surface disinfection by the acid.

ACIDITY AND THE PATHOGENS

The influence of reaction on the growth of *Pythium* and *Rhizoctonia* in culture has been the subject of frequent studies and divergent observations. Various investigators (*9*, *8*, *15*, *1*) report the minimum pH for growth of *Pythium* to be between pH 3.1 and 4.6. Their results indicate the optimum to be between 3.43 and 8.3 and the upper limit between 7.2 and 9.6. *Rhizoctonia* is reported (*15*, *4*, *12*, *13*, *18*) as limited in growth at pH values between 2.4 and 3.8. Optima are given by the several workers as ranging from pH 4.5 to 7 and upper limits from 6.7 to 9.1. Because of this variation it seemed necessary to determine these acidity relations for the fungi already studied intensively.

Potato-dextrose agar was used in these experiments. The medium was autoclaved in 200-ml. lots. Before it had solidified, 20 ml. of M/3 sterilized phosphates (H_3PO_4, NaH_2PO_4, Na_2HPO_4, and Na_3PO_4) in suitable combinations and 0.1 ml. of sterile concentrated sulfuric acid were added to each 200 ml. of medium. The flasks were shaken thoroughly and the agar poured into 13 plates, each containing approximately 15 cc. of agar.

Each of triplicate plates at each reaction was seeded at the center. A 2-mm. disk was used from the advancing margin of a young culture. Four isolates, occupying 12 plates, were studied: *Pythium* F-111-A and F-117 and *Rhizoctonia* F-118 and F-5. At the time of transfer a thirteenth similar plate was heated just to melting and its reaction determined with a glass electrode corrected for temperature. The diameter of each of the colonies was measured every 12 hours, and the vertical and horizontal measurements of each were averaged for a given reading. All cultures were incubated at 24° C.

The results of one experiment in which the 48-hour measurements were plotted are given in figure 7.

Growth of *Pythium* cultures extended from pH 3.7 to about 9 and was most rapid between 5.0 and 8.0. *Rhizoctonia* grew between pH

2.4 and about 9 and most rapidly between 3.5 and 7. Excellent growth for both fungi occurred around pH 6. On relatively acid media, at pH 3 to 4, *Rhizoctonia* grew well, but *Pythium* grew little if at all. However, in media between about 8 and 8.5 *Pythium* grew well while *Rhizoctonia* grew poorly. Growth rate of the two species of *Pythium* was almost identical throughout the range, whereas the rate of growth of the two strains of *Rhizoctonia* differed greatly. Under optimum conditions *Pythium* was inherently faster growing than *Rhizoctonia*.

Only hydrogen-ion concentration was controlled and measured in these investigations. However, as is well known (*14*), very acid media may have a strongly positive Eh and strongly alkaline media a negative Eh. Furthermore, when growth had once started, the metabolic activity of the fungi and atmospheric CO_2 probably influenced the acidity, the oxidation-reduction potential, and various other properties of the medium, which might in turn have affected the growth of the cultures.

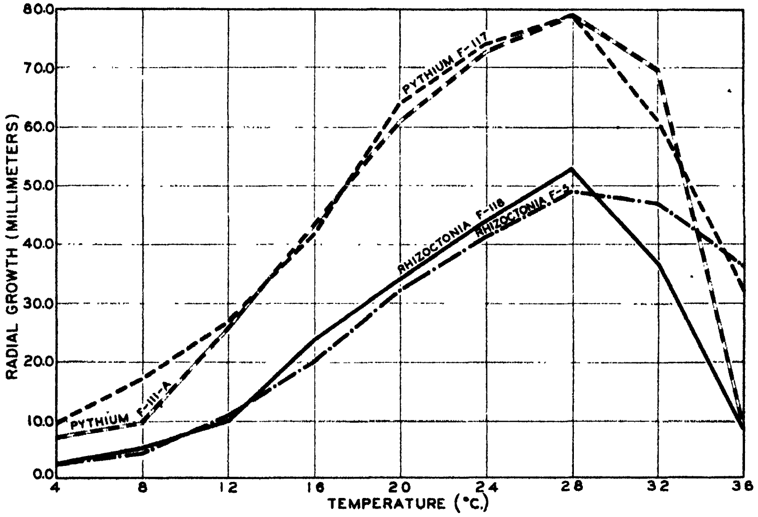

FIGURE 7.—Influence of hydrogen-ion concentration on growth of four damping-off fungi on potato-dextrose agar, adjusted with phosphates over a pH range of 2.3 to 8.9. The values given represent mean measurements in millimeters of two diameters of triplicate colonies at 24° C. 48 hours after inoculation.

ACIDITY AND THE HOST

The influence of soil reaction on host development was observed in the controls of experiments designed for study of the influence of pH on damping-off.

The reaction of the Plainfield sand was adjusted by treating one portion with hydrated lime and another with 3 percent sulphuric acid. The soil was placed out of doors during much of November and December, where it frequently froze and thawed. In the greenhouse the acid and alkaline portions were mixed with untreated soil in various ratios to give a graded series of soil reactions. While this method has some obvious objections, it was used because of the desire to maintain natural conditions as far as possible with respect to other factors involved. After standing for 2 weeks and receiving abundant watering, the different lots of soil were placed in 4-inch pots, autoclaved, and their acidity measured with a glass electrode. A series of nine lots having, respectively, the following reactions was chosen: pH 4.4, 4.9, 5.3, 6.1, 6.6, 7.2, 7.8, 7.9, 8.4. Triplicate pots at each reaction were planted with 25 red pine seeds each and kept as controls. These served for examining the effect of soil reaction on the growth of the host plant. A similar set was inoculated with *Pythium* and a third set with *Rhizoctonia,* as reported in a later section. All the pots were watered, when necessary, with a sprinkling can, and thus the surface had a moisture content varying from about 30 to 100 percent. After 3 weeks under a cheesecloth cover in a 24° C. greenhouse, all seedlings were washed out of the controls, 50 were taken at random from each lot and their root lengths measured.

The optimum range for root growth extended from about pH 4.7 to 6.0 as shown in figure 8, *A*. Growth fell off rapidly at the acid side, but the limit for growth was outside the range used. On the alkaline side growth was relatively poor between about pH 6.0 and 8.0. No top injury was observed in any case, but there was reduced germination (30 percent) at pH 8.5. A second experiment gave comparable data.

These results were in general agreement with those of Wilde (*21*), who found that red pine grew well over a range of pH 5.0 to 6.0.

ACIDITY AND DAMPING-OFF

Detailed investigations of the influence of acidity upon damping-off were made by Hartley (*7*), Jackson (*9*), and C. Roth (*15*), who have reviewed much of the literature. Jackson observed that damping-off of seedlings with *Pythium* and *Rhizoctonia* increased from none at pH 2.5 to a maximum near the neutral point. C. Roth observed the optimum soil reaction for attack by both *Pythium* and *Rhizoctonia* to lie between pH 6.7 and 7.0 with a broad range favorable to growth between pH 5.5 and 8.3. However, with acidity increasing below pH 7.0 the attack by *Pythium* was strongly retarded and was diminished to 0 at pH 4.5. *Rhizoctonia,* on the other hand, caused some damping-off at pH 3.7. Damping-off in general decreased, as Hartley (*7*) observed, with increasing acidity from pH 8.5 to 5.0. He found that, in general, damping-off was not serious in nursery soil more acid than pH 6.0.

The results with Wisconsin materials were secured through the experiment described in the preceding section. Two separate trials gave results which were similar, except that the amount of damping-off was somewhat greater, particularly with *Rhizoctonia,* in the first trial than in the second. The results were averaged for figure 8, *B* and *C*.

A broad reaction range, very favorable for attack by *Pythium,* extended from about pH 5.2 to beyond 8.5. Below 5.2 the amount of damping-off fell rapidly. On the other hand, for *Rhizoctonia* damping-off there appeared a broad range not too favorable for attack between pH 5.2 and 7.8. At the acid end damping-off increased rapidly to a maximum at pH 4.4. The upturn on the alkaline side may arise from the very poor growth of the seedlings near pH 8.2.

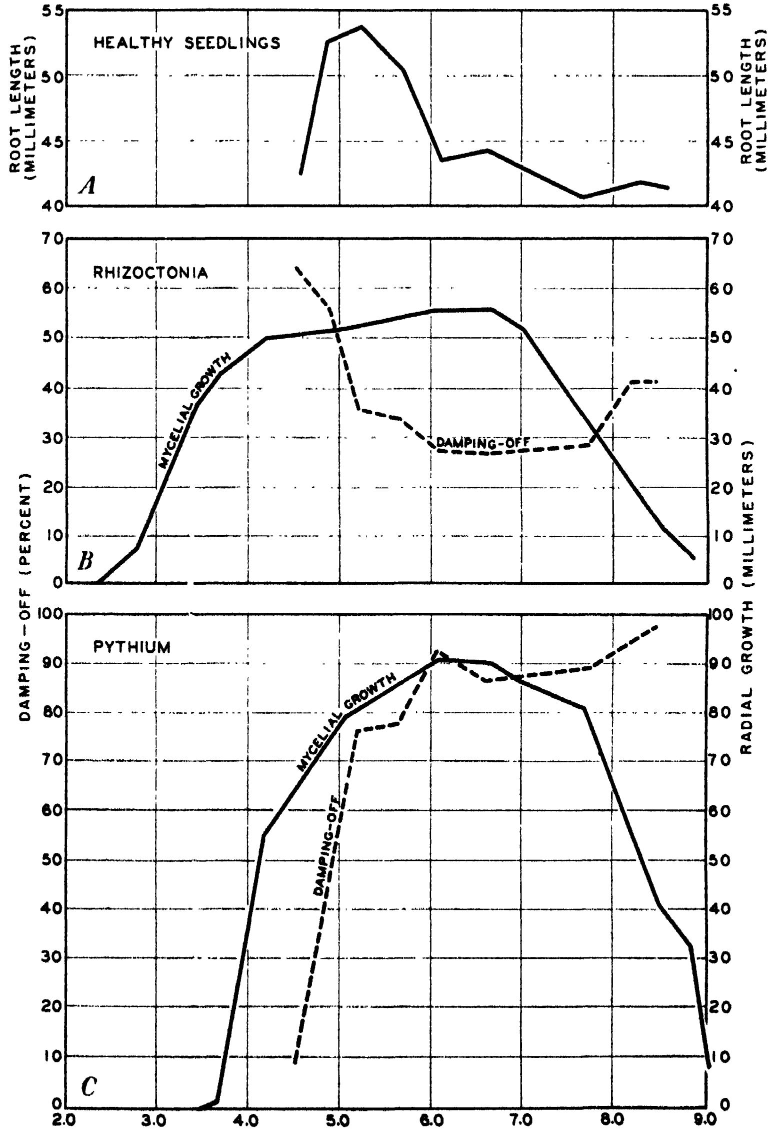

FIGURE 8.—The influence of hydrogen-ion concentration upon: *A*, root length of seedlings; *B*, growth of *Rhizoctonia* in culture and damping-off in soil; *C*, growth of *Pythium* in culture and damping-off in soil.

Increased damping-off by both fungi in soils more alkaline than pH 7.8 appeared correlated with poor host development. Though total damping-off by *Rhizoctonia* was lower than that caused by *Pythium* throughout the range more alkaline than pH 5.2, reaction at no point became a limiting factor.

In juxtaposition to these curves for damping-off (fig. 8, *B* and *C*) are the curves for growth of the fungi in culture at the corresponding reactions. These growth curves were secured by averaging those given in figure 7 for *Pythium* and *Rhizoctonia*, respectively.

The curves representing the growth of *Pythium*, of host plant, and the amount of *Pythium* damping-off (fig. 8, *C* and *A*) roughly correspond in the acid ranges. On the alkaline side *Pythium* damping-off rose while mycelial growth declined. This effect may have been due to reduced growth of the host.

The relation between the curves of damping-off and mycelial growth for *Rhizoctonia* differs markedly from that for *Pythium* (fig. 8, *B*). Reduced damping-off by *Rhizoctonia* and good mycelial growth occurred between about 5.5 and 7.5. Except on the alkaline end, the damping-off data were all taken within the pH range very favorable for growth of *Rhizoctonia*. Damping-off increased with increasing acidity from about pH 6.0. At pH 4.5, where there was still excellent mycelial growth, host development was limited. Consequently, as with *Pythium*, damping-off increased near pH 8.2 though mycelial growth declined.

Soil reaction had no effect upon the ratio of preemergence to postemergence damping-off in these experiments. Wherever damping-off occurred, the loss was approximately equal between these two types.

DISCUSSION

The distinct responses of *Pythium* and *Rhizoctonia* to the influence of temperature, moisture, and soil reaction indicate that damping-off, caused independently by these fungi, may be most wisely considered and treated as two diseases.

Contrary to general concepts, three conditions merit special mention: (1) Damping-off need not necessarily, as its name implies, be a high-moisture disease. *Rhizoctonia* is favored by intermediate moistures and often operates in very dry soil. Excessive soil moisture inhibits this fungus. (2) An acid soil reaction fails as a reliable control for all damping-off. Though acid soil will control damping-off by *Pythium*, it may stimulate that caused by *Rhizoctonia*. However, acid treatment of the soil may have a beneficial effect through partial disinfection of the soil surface. (3) Since the trend of postemergence damping-off is up with rising temperature and since this is the loss commonly seen by nurserymen, it has been concluded that damping-off is a warm-temperature disease. However, at low temperatures there may be excessive *Pythium* losses which go unobserved because they occur before emergence and are thus imputed to poor seed germination.

The present observations have been made under controlled conditions in the greenhouse. The temperature and moisture have been constant at the chosen levels. In nature these factors often fluctuate rapidly and widely. The possibilities are open that the responses of host and parasites might be different in fluctuating environments from those in constant environments. The influence of temperature, moisture, and soil reaction on *Pythium* and *Rhizoctonia* damping-off in the nursery is considered in another paper (*17*).

SUMMARY

Pythium irregulare and *Rhizoctonia solani*, the principal damping-off fungi in Wisconsin forest nurseries (*16*), the diseases they induce, and their host red pine (*Pinus resinosa*) were studied under controlled conditions for their responses to several important environmental factors.

Temperature influenced the rate as well as the amount of seedling emergence. In uninoculated containers germination was poor at 12° and 15° C., good at 18° and 33°, and excellent at from 21° to 30°. The subsequent growth of the seedlings was best between 15° and 30°.

The effect of temperature on damping-off by the two fungi was striking. *Pythium* killed over 90 percent of the seedlings at 12° C., only about half of them at 33°, and had intermediate effects between these temperatures. At 12° *Rhizoctonia* damped-off only a few seedlings. This loss increased to a maximum of approximately 58 percent at 24° to 30° and then declined at 33°.

The optimum temperature for growth of both fungi on potato-dextrose agar was 28° C. At 4° *Rhizoctonia* approached a minimum though *Pythium* continued to grow moderately well. Decline in growth rate above 28° was more rapid for *Pythium* than for *Rhizoctonia*. There appeared to be no relation between rate of growth by *Pythium* in cultures and the amount of damping-off in soil. However, the curve for damping-off by *Rhizoctonia* paralleled that for fungus growth.

With moisture content, *Pythium* damping-off increased from a minimum at 13 percent to a maximum at 100 percent. *Rhizoctonia* damping-off in the soil gradually increased from a high level at 13 percent to a maximum at 68 percent and then decreased to a minimum at saturation.

A successful method for controlling the moisture content of the surface one-half inch of soil, in which damping-off fungi are most active, has been described.

The root lengths of the red pine seedlings were greater in the relatively dry soils than in the wet soils.

Air humidity had little or no influence on *Pythium* damping-off, but high air humidity increased the loss caused by aerial mycelium of *Rhizoctonia*.

Acidity also was important to both mycelial and host growth. In culture, growth of *Pythium* extended from pH 3.7 to about 9 and was best between about 5 and 8. *Rhizoctonia* grew from pH 2.4 to about 9. Most rapid growth was between about 3.5 and 7.5 The optimum range for development of red pine was from about pH 4.7 to 6. Reactions below pH 4.7 and above 6 were relatively toxic.

With respect to damping-off, a broad range favorable for *Pythium* lay between pH 5.2 and 8.5 Below 5.2 the loss was comparatively small. A close relation appeared between the rate of growth in culture and the severity of *Pythium* damping-off. However, for *Rhizoctonia* the range from pH 5.2 to 7.8 was only moderately favorable,

and increase was rapid with acidity stronger than pH 5.2. Increase in damping-off by both fungi at levels above pH 7 appeared associated with decline in host development.

Damping-off by *Pythium* and *Rhizoctonia* was shown to be two distinct diseases.

Information about the influence of environment on the development of these diseases is helpful for accurate interpretation of disease development in the nursery.

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INHERITANCE OF SYMPTOM EXPRESSION OF BEAN MOSAIC VIRUS 4[1]

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INTRODUCTION

Zaumeyer and Harter[2] [3] have recently described a new virus disease of beans (*Phaseolus vulgaris* L.) produced by bean mosaic virus 4 (southern bean mosaic virus 1). Eighty varieties or strains of beans were inoculated and all were found to be susceptible, but the expression of susceptibility varied with the variety used. Twenty-four varieties were homozygous for susceptibility to local lesions, 48 were homozygous for susceptibility to systemic infection, and 8 were heterozygous.

Homozygous varieties or strains that manifested local lesions, although susceptible to the virus at points of inoculation, were immune to the systemic infection. The virus could be recovered from the leaves showing local lesions but not from any other portion of the plant. Local lesions, if they occurred, appeared in 2 or 3 days after inoculation as somewhat circular, brownish-red, necrotic spots that ranged from 1 to 3 mm. in diameter (fig. 1, *A*, *C*). Leaves having numerous necrotic spots dropped off within a short time, thus eliminating the virus. Plants locally infected were susceptible to reinfection.

All the plants that did not show the local-lesion type of infection developed the systemic mottling (fig. 1, *B*, *D*) about 10 days after inoculation. In some varieties the mottling was mild, with little leaf malformation or distortion or plant stunting; in others the leaves were curled and reduced in size, and the plants were stunted. The yield of seed from such plants was much smaller than that from healthy plants. The virus was recovered from all portions of a systemically infected plant, and juice extracted from such a plant always produced necrotic lesions when inoculated to varieties susceptible to the local lesions. No variety was immune to the virus.

The studies reported herein deal with the inheritance of two types of response, as indicated by the segregation of F_2 and F_3 hybrids inoculated with bean mosaic virus 4. These two types of plant response to infection will be referred to throughout the paper as (1) local-lesion infection, in which only local lesions occur on inoculated leaves with no apparent injury to the plant and with no further development of the disease; and (2) systemic-mottle infection, in which mottling and general injury to the plant follow inoculation with a reduction in yield corresponding to the severity of the disease.

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[1] Received for publication November 20, 1942.

[2] ZAUMEYER, W. J., and HARTER, L. L. A NEW VIRUS DISEASE OF BEAN. (Phytopath. Note) Phytopathology 32: 438-439. 1942.

[3] ZAUMEYER, W. J., and HARTER, L. L. TWO NEW VIRUS DISEASES OF BEANS. Jour. Agr. Res. 67: In press. 1943.

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MATERIAL AND METHODS

The virus used in these studies was isolated from infected beans growing under greenhouse conditions at Beltsville, Md.

The crosses were originally made under controlled greenhouse conditions for the study of the inheritance of resistance to another disease; but, when it was noted that one of the parents of each cross was subject only to the local-lesion type of symptoms produced by bean mosaic virus 4 (fig. 1, *A*, *C*), and the other parent showed only the systemic-mottle type of symptoms (fig. 1, *B*, *D*), it was decided to use

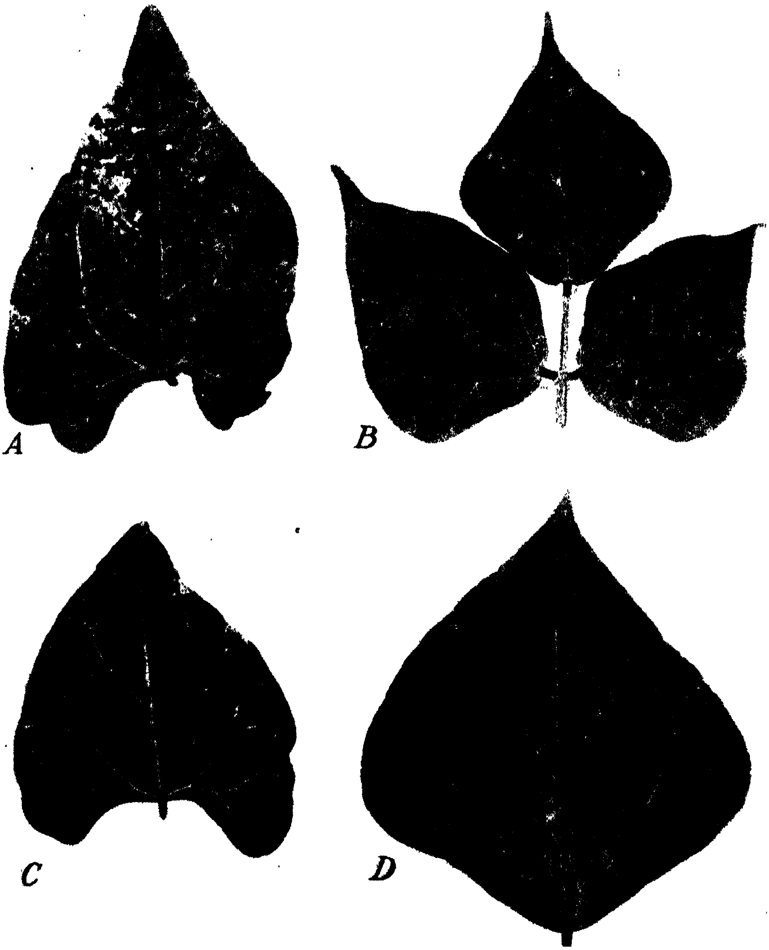

FIGURE 1.—Host reaction to bean mosaic virus 4: *A*, Local necrotic lesions on Pinto (Colorado strain); *B*, systemic mottle on No. 780-2H; *C*, local necrotic lesions on F_2 plant of a cross between Pinto (Colorado strain) and No. 780-2H; *D*, systemic mottle on another F_2 plant of same cross.

this material to determine the mode of inheritance of the expression of symptoms of bean mosaic virus 4. F_1 crosses were not tested. Two of the F_2 crosses were reciprocals of each other (see table 2) and the others were not, but all reacted in a similar manner.

The F_2 plants were inoculated in the greenhouse at Beltsville, Md. The seed was planted in 4-inch pots, and the plants were inoculated when the simple leaves were about three-fourths developed. Carborundum powder No. 38713 was dusted lightly on the leaves, and one simple leaf of each plant was inoculated. This was done in the usual manner by lightly rubbing the leaves with a cheesecloth pad dipped in a slightly diluted extract of the virus. After inoculation the leaves were washed with a fine spray of water. The records of plant response were made about 4 or 5 days after inoculation, and plants on which local lesions were formed and those on which they did not form were labeled and chosen at random for transplanting to greenhouse beds, where they were allowed to grow to maturity. Those that were not transplanted remained in the pots for at least 14 days in order that the number systemically infected might be determined. The number of transplanted plants systemically infected was also recorded. In cases where the mottle symptoms were in doubt, juice was extracted from a few leaves and inoculated to plants of the Ideal Market variety, which is very susceptible to the local-lesion infection. By this method proof was established of the presence or absence of the virus in such plants.

The seed harvested from transplanted F_2 plants was grown in 4-inch pots, and the F_3 plants produced were tested for their reaction to bean mosaic virus 4. In case of doubt as to whether certain F_3 plants were systemically infected, the same procedure was followed as with the F_2 inoculated plants.

All data have been subjected to the χ^2 test for goodness of fit to certain theoretical ratios. This test was applied independently to F_2 families from individual F_1 plants, to F_3 families from F_2 plants, and to the totals of a number of tested families.

EXPERIMENTAL RESULTS

REACTION OF SELFED PARENTS

The reaction to bean mosaic virus 4 of the selfed parents used in the several crosses is shown in table 1. In every case a variety or strain showed either the local-lesion infection or the systemic infection, but never both. Hundreds of plants of commercial varieties were inoculated, and all were found to be susceptible to either local or systemic infection.

TABLE 1.—*Reaction of the selfed parents used in the various crosses when inoculated with bean mosaic virus 4*

Parent	Plants inoculated	Plants showing—	
		Local-lesion infection	Systemic-mottle infection
	Number	*Number*	*Number*
Blue Lake	30	30	0
Brittle Wax	30	0	30
Corbett Refugee (No. 7-B-2)[1]	30	30	0
Cranberry	30	0	30
Pinto (Colorado strain)	30	30	0
Pinto (Idaho strain)	30	30	0
Red Kidney	30	0	30
No. 765 [1] [2]	30	30	0
No. 780-2H [1] [3]	30	0	30

[1] Numbers carried in the files of the writers.
[2] A selection from the Kentucky Wonder Wax variety.
[3] A white-seeded Kentucky Wonder hybrid.

REACTION OF F_2 GENERATION

The F_1 plants were not tested. An analysis of the F_2 data (table 2) shows that the local-lesion type of infection (fig. 1, *A*, *C*) is dominant to the systemic-mottle type (fig. 1, *B*, *D*) with a 3 : 1 segregation, indicating a single-factor difference. The reciprocals behaved alike. The χ^2 values indicated that the observed data fit the calculated quite closely except in 1 case where the χ^2 value exceeded the 5-percent but not the 1-percent point. Out of a total of 450 plants, 335 showed local-necrotic infection and 115 systemic infection. This segregation is a close fit to a 3 : 1 ratio, with a χ^2 value of 0.075.[4]

TABLE 2.—*Reaction of F_2 progenies when inoculated with bean mosaic virus 4*

Percentage of cross	Plants inoculated	Plants showing—		χ^2 for 3 : 1 ratio [1]
		Local-lesion infection	Systemic-mottle infection	
	Number	*Number*	*Number*	
Blue Lake × No. 780-2H[2]	83	66	17	0.904
No. 780-2H[2] × Blue Lake	34	25	9	.038
Brittle Wax × No. 765[2]	147	109	38	.056
Cranberry × Pinto (Idaho strain)	37	27	10	.093
Pinto (Colorado strain) × No. 780-2H[2]	65	41	24	4.928
Red Kidney × Corbett Refugee (No. 7-B-2)[2]	84	67	17	.904

[1] 5-percent point=3.841.
[2] Numbers carried in the files of the writers.

REACTION OF F_3 GENERATION

Of 63 F_3 families derived from F_2 plants that showed the local-lesion type of infection, 24 were homozygous for the local-lesion type of infection and 39 were heterozygous, when inoculated with bean mosaic virus 4 (table 3). The deviation from the calculated 1:ratio of homozygous to heterozygous local-lesion type families was nonsignificant, with a χ^2 value of 0.589.[5] In the 39 heterozygous families, the segregation of plants with the local-lesion type of infection and plants with the systemic-mottle type showed a good fit to a 3 : 1 ratio (table 3). The progenies from 29 F_2 selfed families of the systemic-mottle type of infection were homozygous for systemic infection (table 3). These results contributed additional proof in support of the single-factor hypothesis.

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[4] 5-percent point=3.841.
[5] 5-percent point=3.841.

TABLE 3.—*Reaction of F_3 progenies descended from previously tested F_2 progenies when inoculated with bean mosaic virus 4*

Classification in F_3 generation	Families tested [1]	Plants	Plants showing—	
			Local-lesion infection	Systemic mottle infection
	Number	*Number*	*Number*	*Number*
Homozygous local-lesion type	24	1,065	1,065	0
Heterozygous local-lesion type:				
Observed	39	1,494	1,152	[2] 342
Calculated 3 : 1 ratio	------	------	1,120.5	373.5
Homozygous systemic-mottle type	29	424	0	424

[1] χ^2 for 1 : 2 distribution of families = 0.589; 5-percent point = 3.841.

[2] χ^2 for total population was 3.541; 5-percent point = 3.841. Accumulated χ^2 was 62.547; 5-percent point 62.592 for 39 degrees of freedom.

DISCUSSION

The results clearly demonstrate that in the hybrids investigated the inheritance of the expression of symptoms of bean mosaic virus 4 is governed by a single genetic-factor difference and that the local-lesion infection is dominant to the systemic-mottle type. The reaction of the heterozygous plants was indistinguishable from that of plants homozygous for the local-lesion type of infection.

Holmes,[6] in studying the inheritance of the localization of tobacco mosaic virus in certain pepper varieties, found responses similar to those reported here for beans. His results indicated that, in crosses between pepper varieties that showed only necrotic lesions and varieties that were systemically infected, a single dominant Mendelian factor governed the inheritance of the local-lesion type of infection caused by tobacco mosaic virus. He reported similar results in investigations dealing with the inheritance of resistance to tobacco mosaic in tobacco[7] and *Browallia*.[8]

In the tests here reported a few plants in certain progenies showed the heritable leaf variegation described earlier.[9] The greater the variegation the less chlorophyll the leaves contained. In most instances variegated plants of the dominant genotype did not respond to the local-lesion infection, and, when they did, the lesions were smaller than those on normal plants. On the other hand, the virus was not prevented from becoming systemic in the variegated plants of the recessive genotype. Although the mottled symptoms were difficult to diagnose because of the variegated character, juice from such plants produced local lesions on Ideal Market, proving the presence of the virus. It is not known why local infection was inhibited in the variegated plants and systemic infection was not.

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[6] HOLMES, F. O. INHERITANCE OF ABILITY TO LOCALIZE TOBACCO-MOSAIC VIRUS. Phytopathology 24 : 984–1002, illus. 1934.

[7] ——— INHERITANCE OF RESISTANCE TO TOBACCO-MOSAIC DISEASE IN TOBACCO. Phytopathology 28 : 553–561, illus. 1938.

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[9] ZAUMEYER, W. J. INHERITANCE OF A LEAF VARIEGATION IN BEANS. Jour. Agr. Res. 64: 119–127, illus. 1942.

Immunity to bean mosaic virus 4 has not been observed in any variety thus far tested. Thirty-two varieties have been found that possess the dominant character for virus localization. Twenty-four of these are homozygous for this character and hence are immune to the systemic type of infection. These varieties can be classified as commercially resistant, since little or no damage to the plants results from such infection. Unfortunately most of the important canning and market-garden varieties carry the recessive factor and are therefore subject to the systemic type of infection. Since the inheritance of the types of symptom expression is of a simple nature, little difficulty should be experienced in producing through hybridization varieties susceptible only to local-lesion infection. It would seem that the production of such varieties could eventually eliminate the loss caused by bean mosaic virus 4 in beans. Such varieties are being developed at the present time.

SUMMARY

The inheritance of the expression of symptoms of bean mosaic virus 4 was found to be governed by a single allelomorphic pair of Mendelian factors. Plants carrying the dominant allelomorph are susceptible to a local-lesion type of infection that causes little or no damage, whereas the homozygous recessive plants are susceptible to a systemic type of infection that causes leaf mottling, stunting, and reduction of yield.

Although immunity to bean mosaic virus 4 has not been observed, varieties possessing the dominant gene for virus localization are considered commercially resistant, since in these varieties the infection does not become systemic. Such varieties are being used in hybridization as parental material for producing other commercially resistant varieties.

THE OCCURRENCE OF CITRIC AND ISOCITRIC ACID IN BLACKBERRIES AND IN DEWBERRY HYBRIDS[1]

By A. L. CURL, *assistant chemist*, and E. K. NELSON,[2] formerly *senior chemist, Agricultural Chemical Research Division, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture*

INTRODUCTION

Isocitric acid was first found in nature as the principal acid in eastern blackberries (*9, 11*).[3] Blackberries from the State of Washington were also found to contain isocitric acid (*2*). On the other hand, the principal acid of the Loganberry was found to be citric acid (*8, 10*). These investigations have now been extended to include three named varieties of blackberries, the Brainerd, Crandall (Macatawa), and Texas Wonder, and the dewberry hybrids, Young (Youngberry) and Boysen (Boysenberry).

EXPERIMENTAL METHODS AND MATERIAL

Since there is no published method for the determination of isocitric acid, the ester-distillation method (*3, 4, 5, 6, 7, 9, 11*) which led to the discovery of isocitric acid in blackberries, was used. As this general procedure for the separation of the nonvolatile acids and the fractionation of their ethyl esters has been described previously many details will be omitted here. In this procedure the nonvolatile acids, such as citric, isocitric, and malic, are converted into the ethyl esters, the latter are fractionated, and hydrazides are prepared from the various fractions. The individual acids are identified by the boiling point and optical rotation of their esters, and by the rate and manner of crystallization, the melting point, and the crystalline form of the hydrazides. From the quantities of the various esters obtained, approximate values for the amounts of the corresponding acids present may be calculated.

The fruit used in the investigation was obtained by the Fruit and Vegetable Chemistry Laboratory of the Bureau of Agricultural and Industrial Chemistry in Los Angeles and shipped frozen to Washington, D. C. Table 1 shows the varieties used, the quantities, and the yield of crude ethyl esters.

TABLE 1.—*Fruits examined, quantities used, and yields of crude ethyl esters*

Variety	Quantity of fruit	Crude ethyl esters
	Grams	*Grams*
Brainerd	4,760	52.8
Crandall	8,200	67.5
Texas Wonder	7,860	60.0
Young	6,600	65.4
Boysen	7,060	58.5

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[1] Received for publication October 22, 1942.

[2] Died November 9, 1940.

[3] Italic numbers in parentheses refer to Literature Cited, p., 295.

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Table 2 gives the results of the experiments. All samples were fractionated at 10 mm. Optical crystallographic examinations were made by G. L. Keenan, Food and Drug Administration, Federal Security Agency.

EXPERIMENTAL RESULTS

TABLE 2.—*Physical constants of the fractions of the ethyl esters of designated blackberries and dewberries*

Fraction	Boiling point	Weight	Optical rotation at 20° C.	Melting point of hydrazide	Mixed melting point with known hydrazide
BRAINERD					
	°C.	*Grams*		°C.	°C.
1	84–123	1.8		178	178 *l*-malic hydrazide.
2	123–125	12.9	−11.7	178	178 *l*-malic hydrazide.
3 [1]	175–177	27.0	[2] +5.3	176	
CRANDALL (MACATAWA)					
1	112–126	0.9		178	178 *l*-malic hydrazide.
2	126–130	8.0	−11.4	178	
3 [1]	180–186	51.6	[2] +5.1	176	
TEXAS WONDER					
1	114–125	10.1	−12.0	179	179 *l*-malic hydrazide.
2	125–165	1.6		179	
3	173–176	3.2		168	167 isocitric hydrazide.
[1]	176–181	32.9	[2] −1.2	172	
YOUNG					
1	122–137	4.1	−11.0	179	180 *l*-malic hydrazide.
2	137–170	1.5		177	178 *l*-malic hydrazide.
3 [3]	170–171	46.5		106	
4 [3]	171–181	4.6	[2] −2.0	173	174 isocitric hydrazide.
BOYSEN					
1	Below 118	1.0		178	178 *l*-malic hydrazide.
2	120–130	4.1	−11.3	178	178 *l*-malic hydrazide.
3 [3]	170–175	42.4		105	
4 [2]	Above 175	1.3		172	172 isocitric hydrazide.

[1] Optical crystallographic examination confirmed the identification of the hydrazide as isocitric hydrazide.

[2] The variable values shown are probably due to the formation of a mixture of triethyl isocitrate and diethyl isocitrate lactone from isocitric acid during esterification.

[3] Optical crystallographic examination confirmed the identification of the hydrazides of fractions 3 and 4 as citric and isocitric hydrazides, respectively.

The juice of the Brainerd blackberries was examined for citric acid by the Association of Official Agricultural Chemists' method (*1*).

TABLE 3.—*Proportions of isocitric, citric, and* l*-malic acid found in berries examined*

Variety	Acids		
	Isocitric	Citric	*l*-malic
	Percent	*Percent*	*Percent*
Brainerd	65	0	35
Crandall	85	0	15
Texas Wonder	75	0	25
Boysen	4	85	11
Young	6	86	8

The result (0.05 percent) was practically negative, indicating not more than a trace of citric acid.

The approximate proportions of the isocitric, citric, and *l*-malic acids are given in table 3.

Isocitric acid predominates in the Brainerd, Crandall, and Texas Wonder varieties, all of which are eastern blackberries. Citric acid is the principal acid of the Boysen and Young, and it also occurs almost exclusively in the Logan (Loganberry), all three of which are trailing varieties related to the Pacific coast blackberry. From the above it appears that there may be a relationship between the type of berry and the principal acid (isocitric or citric). No final conclusion should be drawn, however, until a larger number of varieties of both bush and trailing blackberries have been examined.

SUMMARY

The nonvolatile acids present in three blackberries, Brainerd, Crandall, and Texas Wonder, and in two trailing hybrids, the Boysen and the Young, have been investigated by the ester distillation method. The predominating acid in the three blackberries was found to be isocitric acid, whereas in the Boysen and Young dewberries it is citric acid.

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TWO NEW VIRUS DISEASES OF BEANS[1]

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INTRODUCTION

Severely mottled pods of bean (*Phaseolus vulgaris* L.) of unknown varieties were sent to the writers in 1940 and 1941 from Louisiana and California for diagnosis of the mottling, which was suspected of being caused by a virus. Preliminary inoculations of the Ideal Market, Black-Seeded Green Pod, and Full Measure varieties with the expressed juice from the mottled pods from Louisiana produced local lesions in about 3 days on Ideal Market but not on the other two varieties. About 10 days later fairly intense systemic-mottle symptoms appeared on the trifoliate leaves of Full Measure and a milder mottling on Black-Seeded Green Pod. Mottling was never observed on Ideal Market. These tests supported the writer's early belief that a virus not previously described was involved.

Extracted juice from mottled plants of Stringless Green Refugee grown from seed produced the previous year in a screened house at the Bureau of Plant Industry Station, Beltsville, Md., and thought to be infected with bean virus 1, was used to inoculate the same varieties that were inoculated with the previously mentioned virus isolated from the mottled pods, in order to make a preliminary comparison of the two viruses. Three to 4 days after inoculation local lesions appeared on Ideal Market but not on Full Measure and Black-Seeded Green Pod, showing that bean virus 1 was not involved. In some respects the local lesions were not identical with those produced by the virus isolated from the mottled pods. Later mottle symptoms developed on the trifoliate leaves of the last two varieties but not on those of Ideal Market.

This paper deals with the identification of these two viruses and gives descriptions of the diseases produced by them. For the convenience of the reader the names to be applied to the viruses are given here rather than at the close of the paper. The virus isolated from the mottled pods is called bean mosaic virus 4 (southern bean mosaic virus 1) and the virus isolated from the mottled leaves collected in the greenhouse at Beltsville, Md., bean mosaic virus 4A (southern bean mosaic virus 2). It is proposed that according to Holmes' system of classification (*9*),[2] bean mosaic virus 4 be known as *Marmor laesiofaciens* sp. nov. and bean mosaic virus 4A as *Marmor laesiofaciens* var. *minor* var. nov.

DISTRIBUTION

Little is known regarding the distribution of these two viruses under field conditions. Many mottled pods have been collected by market

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[1] Received for publication November 20, 1942.

[2] Italic numbers in parentheses refer to Literature Cited, p. 319.

inspectors from beans originating in Louisiana, and it is likely that both viruses may be rather common in the South. Bean mosaic virus 4A has been isolated from beans grown in Maryland and California, but reports indicate that it was not widespread in those States in 1941. It was also isolated from plants of Stringless Green Refugee grown from seed produced in southern Idaho in 1940. What may be the same virus was brought to the attention of the writers by a seedsman who stated that a large planting of the Golden Gate Wax variety, which is reported to be resistant to bean virus 1, was seriously affected by mosaic in Mississippi in 1941. It is unlikely that the disease in question could have been yellow bean mosaic (bean virus 2), since this virus is not seed-borne in beans and must arise from secondary aphid transmission from mosaic-infected sweetclover growing in proximity to beans. Sweetclover is not commonly grown in the South, and hence it is believed that this virus was not the cause of the disease.

Eight collections of bean mosaic were made in Colorado in 1941 and inoculated to the Ideal Market variety, but in no case was bean mosaic virus 4 or 4A isolated. However, seed grown there in 1941 and planted in the greenhouse at Beltsville, Md., produced mottled plants that were proved to be infected with bean mosaic virus 4A, thus indicating that this virus occurs in Colorado.

METHODS

All of the investigations were conducted under greenhouse conditions at Beltsville, Md., at temperatures ranging from approximately 18° to 27° C. Each virus was studied in a separate unit of the greenhouse, in order to prevent mixture of the viruses. All the plants were inoculated in the early stage of growth. As a rule the inoculations were made on primary leaves 10 to 14 days after planting, but frequently even younger leaves were used. The leaves were dusted lightly with carborundum powder No. 38713, rubbed gently with expressed juice containing the virus, and then, after the mechanical inoculation, washed off with a fine spray of water.

The methods used for studying the properties of the viruses were similar to those employed in previous studies (*24*, *25*).

EXPERIMENTAL RESULTS

FACTORS AFFECTING INFECTION

The present studies showed that infection was more readily obtained with bean mosaic viruses 4 and 4A than with bean virus 1. Even with the use of carborundum powder as an abrasive, 100 percent infection of susceptible varieties was very difficult to obtain with bean virus 1 (*28*). Other investigators (*4*, *12*, *14*) have reported similar results. Although carborundum powder was usually employed in the inoculations, almost as much infection was produced without it. Similarly, leaves sprayed with water after inoculation manifested no more local lesions than unwashed leaves. In practically all inoculations, 100 percent of the plants became infected either locally or systemically.

Infection was readily obtained by painting the simple leaves of Ideal Market with a camel's-hair brush dipped in undiluted expressed juice from plants affected by the two viruses. Within a few days about as many local lesions appeared on those leaves as on the check leaves rubbed in the usual manner. These results indicated the highly infectious nature of the viruses, although the extreme susceptibility of the variety may also have been responsible. With a few exceptions, varieties susceptible to the local-lesion type of infection appeared not to differ in the degree of susceptibility. (See table 1.) Further proof of the infectious nature of bean mosaic virus 4A was demonstrated by brushing the upper sides of 20 leaves of Ideal Market with a camel's-bair brush and then spraying them with a 1 to 50 dilution of the virus. An equal number of unbrushed leaves also were sprayed with the virus of the same dilution. Six days later, 26 local lesions were observed on the brushed leaves and 2 on the unbrushed leaves. Dropping the expressed virus juice on uninjured leaves produced no infection.

Although the age of the inoculated plants did not have as much influence on infection with bean mosaic viruses 4 and 4A as it did on bean virus 1, simple leaves about three-quarters grown were usually chosen for inoculation. In general, old leaves were not as susceptible to infection as young ones.

In order to determine more accurately the relative susceptibility of leaves of different ages, bean mosaic virus 4 was used to inoculate a number of Ideal Market plants 9, 10, 12, 15, 19, 22, and 26 days after planting. On the ninth day the simple leaves were approximately 3 cm. wide, and after 26 days they were approximately mature. The lesions on leaves inoculated 9 and 10 days after planting were well developed but not numerous. After the twelfth- and fifteenth-day inoculations they were very numerous and rather large. Lesions from inoculations after both the nineteenth and twenty-second days were less numerous and smaller. No lesions were produced by inoculations 26 days after planting.

In another experiment one simple leaf about three-quarters grown on each of 20 plants of Ideal Market was inoculated 14 days after planting with a 1 to 50 dilution of bean mosaic virus 4. Six days later the opposite simple leaf was inoculated. At the same time 20 leaves were inoculated on other equal-aged plants on which neither of the primary leaves had previously been inoculated. The total number of local lesions on the first inoculated leaves was 1,348, while the total number of lesions on the opposite leaves, which were inoculated 6 days later, was 536. The number of lesions on the third set of inoculated plants was 484. Besides being fewer in number, the lesions were smaller and slower to appear than those on the first inoculated leaves.

It was also demonstrated experimentally that fewer lesions were produced on old simple leaves than on young trifoliate leaves of the same plant inoculated at the same time. The lesions produced on old simple leaves were usually smaller than those on immature simple leaves.

Whether local or systemic symptoms were produced by bean mosaic virus 4 and bean mosaic virus 4A depended on the variety inoculated. On such varieties as Alabama No. 1, Blue Lake, Pink, and Ideal Market, only local lesions were produced. (See table 1.) On Bountiful, Commodore, Full Measure, and Brittle Wax, only the systemic mottle symptoms were produced. (See table 1.) In no case did the same plant of any variety thus far tested show both local and systemic infection. In a few instances mottle symptoms appeared on certain plants that had exhibited local lesions on the inoculated leaves, but later such mottling was found to be caused by bean virus 1. Since bean virus 1 is seed-borne, it is not likely that the symptoms would be noticed in all cases on the primary leaves; in some cases they would be noted only on the trifoliate leaves.

Occasionally only a few lesions were produced on an inoculated leaf, but in no such instance did the trifoliate leaves show mottle symptoms. Similarly, in a few instances varieties normally showing local lesions manifested none at all upon inoculation, but in no case did they thereafter exhibit mottle symptoms; that is, they simply escaped infection.

When it was repeatedly noted that a plant that exhibited local lesions never manifested systemic infection, a mixture of two viruses was suspected in case of both bean mosaic virus 4 and bean mosaic virus 4A—one producing only local lesions and the other only systemic infection; it was also surmised that possibly the local lesions immunized the plant against systemic infection. However, varieties susceptible to the local-lesion type of infection when inoculated with expressed juice from mottled leaves of plants inoculated with either bean virus 4 or bean virus 4A produced local lesions only, indicating that only a single virus was involved. It is quite conceivable from a genetic standpoint that a plant is resistant or susceptible either to the local or to the systemic infection. In most cases (see table 1) the varieties were homozygous for either local-lesion or systemic infection. A few varieties, as, for example, Davis Stringless Wax, French Horticultural, Low Champion, and Idaho Refugee, were heterozygous. (See table 1.)

SYMPTOMS

Bean Mosaic Virus 4

The local lesions produced by bean mosaic virus 4 on a variety such as Ideal Market frequently appear about 3 days after inoculation and even earlier under ideal conditions. Usually they are almost circular in shape, brownish red in color, and frequently have light centers. They range from 1 to 3 mm. in diameter. The size of the lesions depends in part on the variety and the age of the plant when inoculated and in part on the number of lesions per unit area. On certain varieties such as Pink the lesions may attain a diameter of 3 to 4 mm. (fig. 1, *A*). On most varieties the lesions do not have a clearly defined edge but are somewhat diffuse or spreading (fig. 1, *A* and *B*). When the virus is very concentrated, the lesions may be so numerous that they coalesce, often causing the leaf to die and drop off. When the lesions are located near the veins and veinlets, the tissue may become necrotic for a distance of 1.5 to 3 cm. from the point of infection (fig. 1, *A* and *B*).

The systemic or mottled symptoms produced by bean mosaic virus 4 are noted only on those plants that do not exhibit local lesions. The first evidence of infection is a mild mottle of the trifoliate leaves (fig. 2, *C* and *E*) similar to that produced by bean virus 1. Later, especially on those varieties that are somewhat tolerant to bean virus 1, such as Full Measure, Burpee Stringless Green Pod, Brittle Wax, and Hardy Wax, the mottling becomes quite intense. Vein banding is common, the interveinal tissue being lighter green than the tissue adjacent to the veins. The leaves may also be puckered and blistered, typical of the symptoms produced by bean virus 1 (fig. 2, *B*) as they occur on Stringless Green Refugee and other highly susceptible varieties. Bean mosaic virus 4 produces very mild symptoms that can

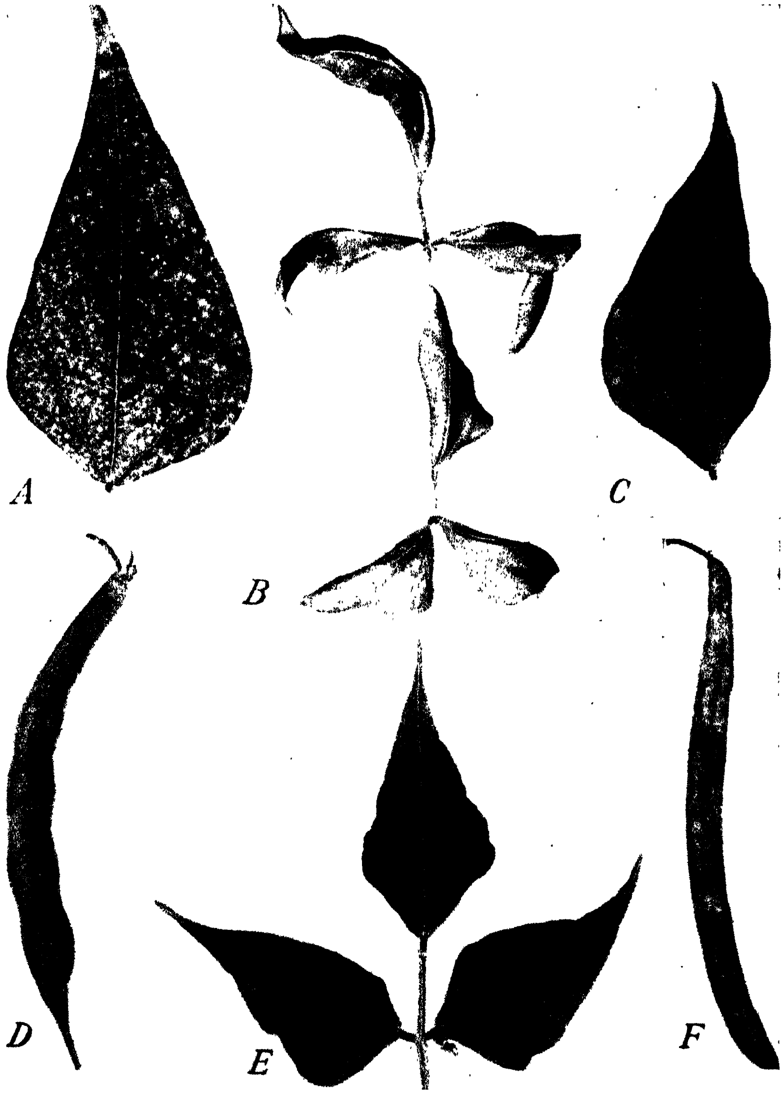

FIGURE 1.—Local lesions produced by bean mosaic viruses 4 and 4A on several varieties: *A* and *B*, Bean mosaic virus 4 on Pink and Pinto (Colorado strain), respectively; *C* and *D*, bean mosaic virus 4A on Ideal Market and Low Champion, respectively. *E*, Leaf of Full Measure inoculated with bean mosaic virus 4, on which neither virus causes local lesions. *F*, Uninoculated leaf of Ideal Market.

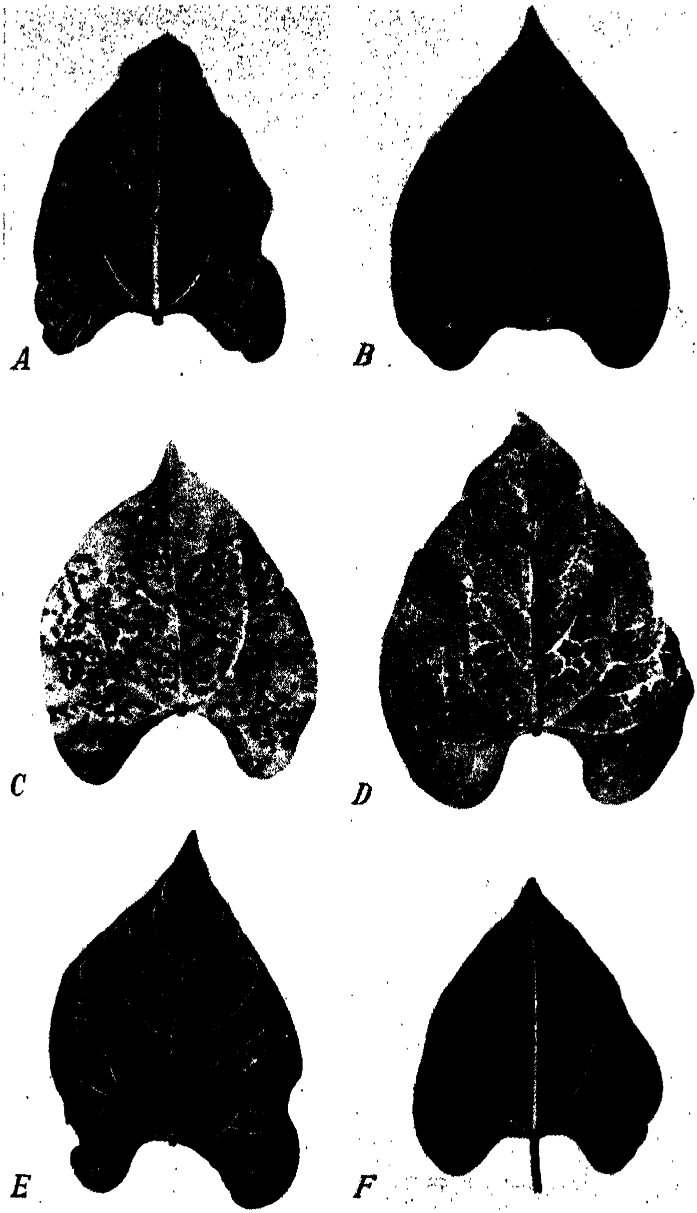

FIGURE 2.—Variations in systemic symptoms produced by several viruses infectious to bean: Stringless Green Refugee leaves infected with bean virus 2 (*A*), bean virus 1 (*B*), and bean mosaic virus 4 (*C*); Stringless Green Refugee pod infected with bean virus 1 (*D*); U. S. No. 5 Refugee leaf infected with bean mosaic virus 4 (*E*); Black Valentine pod infected with bean mosaic virus 4 (*F*).

be readily overlooked on Stringless Green Refugee (fig. 2, *C*) as well as on the mosaic-resistant Refugee varieties (fig. 2, *E*). (See table 1.) On some varieties, as, for example, Low Champion and Red Valentine, vein necrosis, which may cause the leaves to drop off, occurs on the young trifoliate leaves. Reduction in leaf size and malformation occur on very susceptible varieties. In general, it is often difficult to differentiate bean virus 1 from bean mosaic virus 4 by the systemic symptoms alone.

The symptoms on the pods produced by bean mosaic virus 4 are more marked than those caused by any other virus thus far reported as infectious to beans. They appear as dark-green, irregularly shaped, water-soaked-like, blotched areas (fig. 2, *F*) on the green-podded types and as greenish-yellow areas on the wax-podded types. These areas suggest a condition often caused by low temperatures. Infected pods of susceptible varieties are slightly malformed, subnormal in length, and frequently curled at the end, owing to improper ovule development. Under greenhouse conditions, the symptoms are not as marked as they are under ideal field conditions.

BEAN MOSAIC VIRUS 4A

As with bean mosaic virus 4, two types of symptoms are produced by bean mosaic virus 4A. The local lesions are very similar to those just described for bean mosaic virus 4 except that they are less diffuse and spreading and the edges are more distinct (fig. 1, *C* and *D*). In other respects they cannot be differentiated from those produced by bean mosaic virus 4.

In general the systemic symptoms are not as severe in the early stages as those produced by bean mosaic virus 4, but in the later stages they are more severe; much stunting, curling, malformation, and reduction in size of leaves occur (fig. 3, *B*, *C*, *E*, and *H*). It is difficult to differentiate these two viruses by symptomatology alone except on certain varieties. They can, however, be differentiated from the symptoms produced by bean virus 1 (fig. 2 *B*) on some varieties where the symptoms of this virus are very well known. Varietal differences alter the expression of the symptoms.

SUSCEPTIBILITY TESTS

REACTION OF BEAN VARIETIES

Eighty varieties or strains of beans (*Phaseolus vulgaris*) were inoculated with bean mosaic viruses 4 and 4A. Since the results with both were quite similar only those with bean mosaic virus 4 are given in table 1. Twenty-four varieties were homozygous for susceptibility to local-lesion infection of bean mosaic virus 4, 48 were homozygous for susceptibility to systemic infection, and 8 were heterozygous. None of the varieties that were 100 percent susceptible to local lesions showed systemic infection. Among the varieties homozygous for systemic infection, 13 exhibited severe symptoms, 8 were moderately infected, and 27 were mildly affected.

Thirty varieties were susceptible to the local lesions of bean mosaic virus 4A, and 6 of these were heterozygous. Five of these, namely, French Horticultural, White Kentucky Wonder (white-seeded,

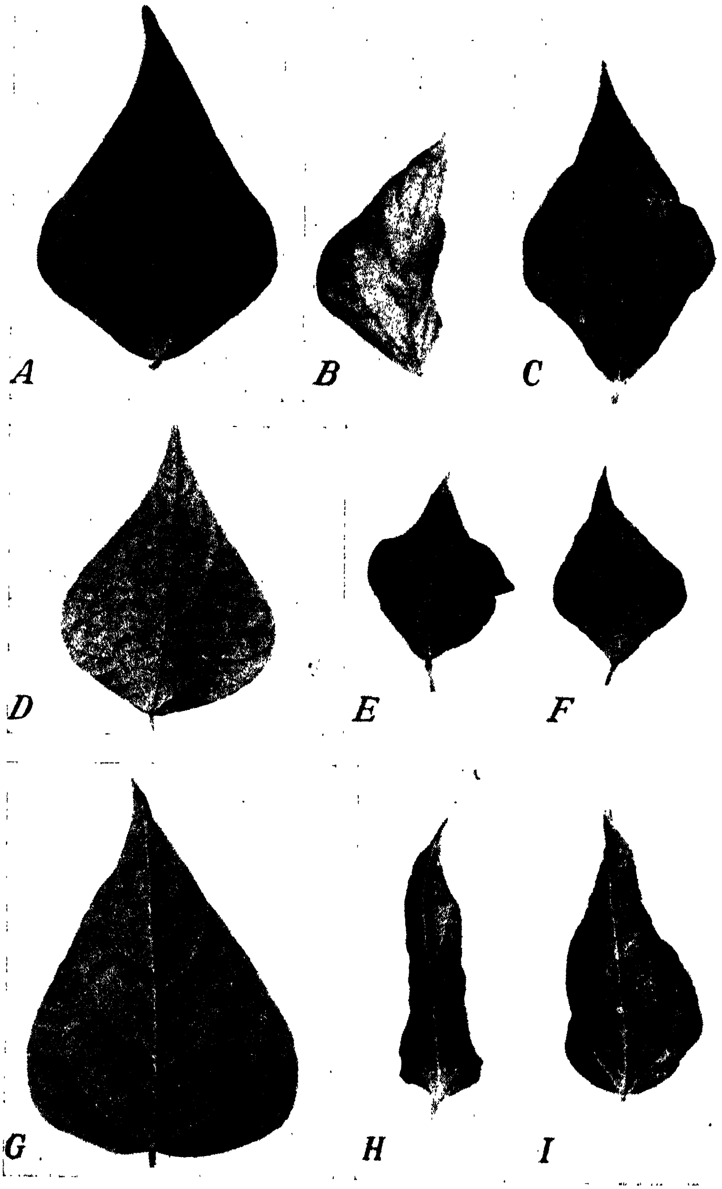

FIGURE 3.—Variations in systemic-mottle symptoms produced by bean mosaic viruses 4 and 4A on several varieties: Large White Marrow infected with bean mosaic virus 4 (*A*) and with bean mosaic virus 4A (*B* and *C*); Webber Wax infected with bean mosaic virus 4 (*D*) and with bean mosaic virus 4A (*E* and *F*); Dixie White infected with bean mosaic virus 4 (*G*) and with bean mosaic virus 4A (*H* and *I*).

TABLE 1.—*Reaction of bean varieties to inoculation with bean mosaic virus 4 compared with reaction to bean virus 1*

Group and variety or strain	Bean mosaic virus 4				Degree of systemic infection with bean virus 1[1]
	Plants inoculated	Plants showing—		Degree of systemic infection	
		Local lesions	Systemic infection		
	Number	*Number*	*Number*		
Green-podded garden beans (bush):					
Asgrow Stringless Green Pod	10	0	10	Mild	Mild.
Black-Seeded Green Pod	11	0	11	do	Do.
Bountiful	10	0	10	Moderate	Do.
Burpee Stringless Green Pod	10	0	10	Mild	Do.
Commodore	9	0	9	Severe	Do.
Dixie White	10	0	10	Mild	
Early Mohawk	8	0	8	do	Moderate.
French Forcing	11	0	11	Moderate	
Full Measure	12	0	12	Severe	Mild.
Giant Stringless Green Pod	10	0	10	Mild	Do.
Goddard (Boston Favorite)	10	0	10	do	Do.
Horticultural, Dwarf	12	0	12	Severe	Moderate.
Landreth Stringless Green Pod	10	0	10	Mild	Mild.
Longfellow	10	0	10	do	Do.
Low Champion	19	6	13	Severe[2]	Moderate.
Plentiful	10	0	10	Moderate	Mild.
Refugee, Corbett	10	10	0		Resistant.
Refugee, Idaho	11	4	7	Mild	Do.
Refugee, Medal	10	0	10	do	Do.
Refugee, Sensation No. 1066	10	0	10	do	Do.
Refugee, Sensation No. 1071	10	0	10	do	Do.
Refugee, Stringless Green	13	0	13	do	Severe.
Refugee, U. S. No. 5	11	0	11	do	Resistant.
South America No. 1	10	0	10	do	
Tennessee Green Pod	11	11	0		Moderate.
Valentine, Asgrow Black	11	0	11	Moderate	Mild.
Valentine, Black	10	0	10	do	Do.
Valentine, Red	10	0	10	Severe[2]	Severe.
Wax-podded garden beans (bush):					
Brittle Wax (Round Pod Kidney Wax)	10	0	10	Severe	Mild.
Davis Stringless Wax	10	4	6	Mild	Do.
Golden Eye Wax	10	0	10	Severe	
Hardy Wax	11	0	11	do	Mild.
Hodson Wax	9	0	9	do	Severe.
Improved Golden Wax	11	0	11	Mild	Moderate.
Improved Stringless Kidney Wax	10	0	11	Severe	Mild.
Pencil Pod Black Wax	11	1	10	Moderate	Moderate.
Prolific Black Wax	8	0	8	Mild	Mild.
Sure Crop Wax	12	0	12	Severe	Moderate.
Top Notch Golden Wax	10	0	10	Mild	
Unrivalled Wax	10	0	10	do	Mild.
Wardwell Kidney Wax	10	0	10	Moderate	Do.
Webber Wax	10	0	10	Mild	Do.
Green-podded garden beans (pole):					
Alabama No. 1	10	10	0		
Black Climbing	10	0	10	Mild	
Blue Lake (White Creaseback)	12	12	0		Do.
Cranberry	11	0	11	Mild	Do.
Cutshort (Corn Hill)	10	10	0		
Decatur	10	10	0		Resistant.
Dutch Caseknife	10	10	0		
Horticultural, French	6	1	5	Mild	Moderate.
Horticultural, London	10	0	10	Severe	Severe.
Horticultural, Mammoth	12	0	12	Mild	Do.
Ideal Market	40	40	0		Mild.
Kentucky Wonder	12	12	0		Do.
Kentucky Wonder, White (Burger Stringless).	9	9	0		
Kentucky Wonder, White (rust-resistant)	9	6	3	Mild	Do.
Kentucky Wonder (Morse 191)	12	12	0		
Kentucky Wonder No. 780[3]	14	14	0		
Kentucky Wonder No. 814[3]	9	9	0		
Kentucky Wonder, U. S. No. 3	12	12	0		Do.
Lazy Wife (White Cranberry)	11	0	11	Moderate	Severe.
McCaslan	10	10	0		
Missouri Wonder	11	11	0		
Oregon Giant	9	9	0		
Striped Creaseback (Scotia)	9	5	4	Mild	
White Half Runner	10	2	8	Moderate	

See footnotes at end of table.

TABLE 1.—*Reaction of bean varieties to inoculation with bean mosaic virus 4 compared with reaction to bean virus 1*—Continued

Group and variety or strain	Bean mosaic virus 4				Degree of systemic infection with bean virus 1[1]
	Plants inoculated	Plants showing—		Degree of systemic infection	
		Local lesions	Systemic infection		
	Number	*Number*	*Number*		
Wax-podded garden beans (pole):					
Golden Cluster Wax	11	0	11	Mild	Severe.
Golden Gate Wax	10	0	10	Severe	Resistant.
Kentucky Wonder Wax	10	10	0		Mild.
Kentucky Wonder Wax No. 765[3]	11	11	0		Moderate.
Green-podded field beans (bush):					
Burnley	8	0	8	Severe	
Marrow, Large White	11	0	11	Mild	
Pink	11	11	0		Mild.
Red Kidney	12	0	12	Moderate	Moderate.
Green-podded field beans (pole):					
Great Northern U. I. No. 59	12	12	0		Resistant.
Pinto, Colorado strain	12	12	0		Moderate.
Pinto, Idaho strain	10	10	0		Do.
Red Mexican U. I. No. 34	10	10	0		Resistant.
Robust	10	0	10	Mild	Do.
Small White (California strain)	10	0	10		Mild.

[1] Data recorded from earlier results of writers as well as published results of other investigators.

[2] Trifoliate necrosis.

[3] Kentucky Wonder types; numbers carried in the files of the writers.

rust-resistant), Low Champion, Idaho Refugee, and White Half Runner, were also heterozygous for susceptibility to bean mosaic virus 4. Davis Stringless Wax, Pencil Pod Black Wax, and Striped Creaseback were resistant to the local lesions of bean mosaic virus 4A but were heterozygous for bean mosaic virus 4. Small White (California strain), on the other hand, did not express local lesions with bean mosaic virus 4, but 1 of 10 plants inoculated with bean mosaic virus 4A produced local lesions.

Fifty varieties were totally susceptible to the systemic infection with bean mosaic virus 4A; 6 of these were heterozygous; as was the case with bean mosaic virus 4, those varieties that were not locally infected were susceptible to systemic infection. Of the varieties homozygous for susceptibility to systemic infection, 8 were severely infected, 22 moderately, and 20 mildly. In general, the symptoms produced by bean mosaic virus 4A are somewhat more severe than those produced by bean mosaic virus 4 (fig. 3).

Certain varieties resistant to bean virus 1, namely, Corbett Refugee, Great Northern U. I. No. 59, and Red Mexican U. I. No. 34, were homozygous susceptible to the local lesions produced by both viruses, while Idaho Refugee was heterozygous (table 1). U. S. No. 5 Refugee (fig. 2, *E*), Sensation Refugee Nos. 1066 and 1071, Medal Refugee, and Robust were resistant to bean virus 1 and the local-lesion type of infection by bean mosaic viruses 4 and 4A but were susceptible to systemic infection by the last two.

Table 1 shows that in general the varieties tolerant to bean virus 1, as, for example, Bountiful, Full Measure, Improved Stringless Kidney Wax, and Brittle Wax, were very susceptible to the systemic infection of bean mosaic virus 4; these varieties were also very susceptible to bean mosaic virus 4A. In a few cases varieties very susceptible to bean virus 1, such as Golden Cluster Wax, Mammoth Horticultural, Lazy Wife, and especially Stringless Green Refugee, were less susceptible to the systemic infection of bean mosaic viruses 4 and 4A. Hodson Wax, London Horticultural, and Red Valentine were very susceptible to the systemic infection by the three viruses.

There seems to be some relation between the pole habit of growth and susceptibility to local lesions and between the bush habit of growth and susceptibility to systemic infection. Twenty-one pole varieties were homozygous susceptible to local lesions caused by bean mosaic virus 4 and 9 did not express them, while only 3 bush varieties were homozygous susceptible to local infection and 39 were homozygous susceptible to systemic infection. Four pole and four bush varieties were heterozygous. All the pole Kentucky Wonder strains were susceptible to local lesions except White Kentucky Wonder (rust-resistant), which was heterozygous, while Commodore, a Kentucky Wonder bush type, was resistant to local infection.

Of 18 wax-podded varieties tested with bean mosaic virus 4, 2 (pole types) were homozygous susceptible to local infection only, 14 did not express local-lesion symptoms, and 2 were heterozygous. Of 62 green-podded varieties, 22 were homozygous susceptible to local lesions, 34 homozygous resistant, and 6 heterozygous.

REACTION OF LIMA BEANS

Ten plants each of 10 varieties of lima beans were inoculated with bean mosaic viruses 4 and 4A. Five small-seeded varieties, Baby Fordhook, Baby Potato, Henderson Bush, Illinois Large Podded, and Wood Prolific, were 100 percent susceptible to only the local-lesion type of infection of both viruses. The first three varieties are not true sieva types (*Phaseolus lunatus* L.) but are hybrids between a small sieva and a large-seeded Fordhook (*P. lunatus macrocarpus* Benth.). The following large-seeded varieties were immune to both viruses: Burpee Bush, Burpee Improved Bush, Fordhook Bush, Fordhook (Asgrow strain), and Philadelphia Bush.

REACTION OF OTHER HOSTS

In order to ascertain the host range of bean mosaic viruses 4 and 4A, a number of plant species were chosen and mechanically inoculated. Ten plants of each of 31 species, representing 20 genera in 5 families, were inoculated. Only *Soja max* var. Virginia was susceptible to both viruses. The symptoms were noted as a very mild mottling of the leaves. No infection, either local or systemic, was observed on any of the following plants tested:

Chenopodiaceae:
  Beet, *Beta vulgaris* L. var. Detroit Dark Red.
Cruciferae:
  Turnip, *Brassica rapa* L. var. Purple Top White Globe.
Cucurbitaceae:
  Cucumber, *Cucumis sativus* L. var. White Spine.

Leguminosae:
  Chickpea, *Cicer arietinum* L.
  Crotalaria, *Crotalaria spectabilis* Roth.
  White lupine, *Lupinus albus* L.
  Yellow sweet lupine, *L. luteus* L.
  Alfalfa, *Medicago sativa* L.
  White sweetclover, *Melilotus alba* Desr.

Leguminosae—Continued.
- Adzuki bean, *Phaseolus angularis* (Willd.) W. F. Wight.
- Mung bean, *Phaseolus aureus* Roxb.
- Scarlet runner bean, *Phaseolus coccineus* L.
- Urd bean, *Phaseolus mungo* L.
- Pea, *Pisum sativum* L. var. Wisconsin Early Sweet.
- Soybean, *Soja max* (L.) Piper var. Biloxi.
- Velvetbean, *Stizolobium deeringianum* Bort.
- Crimson clover, *Trifolium incarnatum* L.
- Red clover, *Trifolium pratense* L.
- White clover, *Trifolium repens* L.
- Broadbean, *Vicia faba* L.
- *Vicia cylindrica* L.

Leguminosae—Continued.
- Common vetch, *Vicia sativa* L.
- Hairy vetch, *Vicia villosa* Roth.
- Asparagus-bean, *Vigna sesquipedalis* (L.) Fruwirth.
- Cowpea, *V. sinensis* (Torner) Savi var. Groit.

Solanaceae:
- Pepper, *Capsicum annum* L. var. World Beater.
- Jimsonweed, *Datura stramonium* L.
- Tomato, *Lycopersicon esculentum* Mill. var. Marglobe.
- *Nicotiana glutinosa* L.
- Tobacco, *Nicotiana tabacum* L. var. Turkish.
- Petunia, *Petunia hybrida* Vilm. var. Rosy Morn.

RELATION OF TEMPERATURE TO SYMPTOM EXPRESSION

In order to ascertain the influence of air temperatures on the expression of local and systemic symptoms, 8 groups of 10 plants each of Ideal Market and Brittle Wax were inoculated in the usual manner with bean mosaic viruses 4 and 4A. Ten plants of each variety were placed in sections of the greenhouses maintained at day temperatures of approximately 16°, 18°, 24°, and 27° C. In the greenhouse held at 27°, the night temperatures were slightly lower than the day temperatures.

Bean mosaic virus 4 produced local lesions on Ideal Market in 3 days at 27°, 4 days at 24°, 5 days at 18°, and 6 days at 16° C. However, the symptoms were not clearly defined at the lowest temperature until the seventh day. Bean mosaic virus 4A produced local lesions in 3 and 4 days, at 27° and 24°, respectively, in 6 days at 18°, and in 7 days at 16°. Symptom expression was not clear until the twelfth day after inoculation. At 27° and 24°, the local lesions produced by both viruses were more numerous but smaller than at the two lower temperatures.

The initial systemic symptoms caused by bean mosaic virus 4 on Brittle Wax appeared in 8 days at 27°, 24°, and 18°, and in 13 days at 16° C. At the end of 20 days fairly distinct mottling was observed at 27°, good at 24°, very good at 18°, and good to very good at 16°. The initial systemic symptoms caused by bean mosaic virus 4A appeared in 7 days at 27° and 18°, in 5 days at 24°, and in 12 days at 16°. At the end of 20 days, distinct mottling was noted at 27°, very good at 24° and 18°, and fairly good at 16°.

In general, the systemic symptoms produced by bean mosaic virus 4 are less intense than those produced by bean virus 4A. This difference is one of the characteristics distinguishing them (fig. 3). The experiments just reported prove that the most severe symptoms are produced by bean mosaic virus 4 at 18° C. and by bean mosaic virus 4A at 18° and 24°.

SEED TRANSMISSION

Extracts of bean mosaic viruses 4 and 4A from seed in the milk and dough stages and from newly ripened seed of systemically infected plants produced excellent infection on Ideal Market. Extracts from seed produced on plants systemically infected with bean mosaic virus 4 and stored for 7 months produced no infection on Ideal Market, but extracts from similarly aged seed from plants infected with bean mosaic virus 4A produced a few local lesions on that variety.

Seed from the two lots that had been aged 7 months was planted in the greenhouse. Juice extracted from the plants produced by seed borne on plants systemically infected with bean mosaic virus 4 did not cause infection of Ideal Market plants, but juice from plants produced by seed of plants infected with bean mosaic virus 4A caused local infection of inoculated Ideal Market plants. The leaf symptoms on such infected plants were very mild, and in some cases infected plants were almost indistinguishable from normal ones; this made it difficult to determine the exact percentage of infected plants. Of 200 plants about 5 percent were infected.

DISTRIBUTION OF THE VIRUSES IN INFECTED PLANTS

Inoculations with juice expressed from the root, hypocotyl, epicotyl, leaf, and pod tissues of plants systemically infected with both viruses produced local lesions on Ideal Market. Calculated on the basis of lesions produced, the viruses were slightly less concentrated in the root than in other tissues. Fajardo (*6*) was unable to isolate bean virus 1 from the roots of infected plants.

When separate extracts were made from the seed coats, cotyledons, and embryos of a number of green seed infected with both viruses and Ideal Market plants were inoculated, local lesions were produced in every case. The viruses appeared to be the most concentrated in the seed coat.

PROPERTIES OF THE VIRUSES

Extracts from newly infected bean plants exhibiting systemic symptoms were used throughout the experiment, and the results were based on local lesions produced on Ideal Market.

THERMAL INACTIVATION

The thermal inactivation points for bean mosaic viruses 4 and 4A were found to be about 95° C. after heating for 10 minutes in sealed thin-walled glass tubes in a water bath (table 2). This temperature

TABLE 2.—*Comparison of thermal inactivation points of bean mosaic viruses 4 and 4A, as determined by production of local lesions on the Ideal Market variety*

Temperature (° C.)	Reaction to—							
	Bean mosaic virus 4				Bean mosaic virus 4A			
	Trial 1		Trial 2		Trial 1		Trial 2	
	Leaves inoculated	Total lesions	Leaves inoculated	Total lesions	Leaves inoculated	Total lesions	Leaves inoculated	Total lesions
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Not heated	20	6,000–8,000	20	4,000–5,000	20	6,000–8,000	20	4,000–5,000
50	20	8,000	----------	----------	20	8,000	----------	----------
80	22	3,300	----------	----------	24	3,450	----------	----------
85	24	348	26	2	24	343	18	106
90	22	9	26	0	22	1	22	58
95	22	0	26	1	22	0	21	7

is higher than that for any other legume virus thus far described and approximates that of tobacco mosaic virus 1, which is inactivated at 93°, and that of cucumber viruses 3 and 4, which are inactivated at 80° (*1*). Bean virus 1, according to previous studies (*2*, *14*, *28*), is inactivated between 56° and 58°. The highest thermal inactivation point for any of the previously described legume viruses is for alfalfa mosaic virus 1 and its strains (*14*, *24*), which lose their infectivity between 65° and 75°.

TOLERANCE TO DILUTION

Bean mosaic viruses 4 and 4A withstood dilutions of 1 to 500,000 (table 3), much higher than bean virus 1, which has a dilution end point of 1 to 2,000. Three legume viruses have been reported to be infectious at high dilutions. These are pea virus 2, described by Osborn (*13*), and severe pea mosaic, described by Johnson and Jones (*11*), which have been reported to be infectious up to 1 to 100,000 dilution, and pea streak virus reported by Chamberlain (*5*) to be infectious at a 1 to 1,000,000 dilution. In no other respects do these three viruses show any relationship to bean mosaic viruses 4 and 4A.

TABLE 3.—*Comparison of tolerance to dilution of bean mosaic viruses 4 and 4A, as determined by production of local lesions on the Ideal Market variety*

Dilution	Reaction to—							
	Bean mosaic virus 4				Bean mosaic virus 4A			
	Trial 1		Trial 2		Trial 1		Trial 2	
	Leaves inoculated	Total lesions	Leaves inoculated	Total lesions	Leaves inoculated	Total lesions	Leaves inoculated	Total lesions
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
None	20	4,000-5,000±	20	4,000-5,000±	20	5,000-6,000±	20	4,000-5,000±
1 to 1,000	20	5,000±	20	5,000±	20	6,000±	20	5,000±
1 to 50,000	20	158	--------	--------	20	165	--------	--------
1 to 100,000	20	131	22	47	20	193	21	95
1 to 500,000	--------	--------	18	25	--------	--------	22	19

AGING

Infection with bean mosaic viruses 4 and 4A was obtained after the extracted juice was held in vitro at 18° C. for 32 weeks (table 4).

TABLE 4.—*Comparison of resistance to aging in vitro of bean mosaic viruses 4 and 4A (at 18° C.), as determined by production of local lesions on the Ideal Market variety*

Time aged (weeks)	Reaction to—					
	Bean mosaic virus 4			Bean mosaic virus 4A		
	Plants inoculated	Plants infected	Local lesions	Plants inoculated	Plants infected	Local lesions
	Number	*Number*		*Number*	*Number*	
None	10	10	Abundant	10	10	Abundant.
1	10	10	do	10	10	Do.
12	--------	--------	--------	10	10	Do.
18	10	10	Good	--------	--------	
24	--------	--------	--------	10	8	Medium.
32	10	1	Very few	10	10	Do.

Only a few local lesions were produced by bean mosaic virus 4 after this length of time, whereas bean mosaic virus 4A produced a fairly large number of lesions. No other legume virus thus far investigated has resisted aging in vitro for this length of time. Bean virus 1 (*14*, *28*) loses its infectivity in vitro after 32 hours. Severe mosaic of pea, according to Johnson and Jones (*11*), was infectious for 15 days.

INACTIVATION BY CHEMICALS

Since bean mosaic viruses 4 and 4A reacted very similarly to thermal inactivation, dilution, and aging in vitro, only the former was tested with chemicals. The treatments lasted 30 minutes. The results (table 5) show that bean mosaic virus 4 is more tolerant to chemicals than bean virus 1. According to earlier studies (*6*, *14*, *28*), bean virus 1 was inactivated in 50 percent alcohol in 30 minutes, whereas bean mosaic virus 4 was highly infectious after being exposed to 95 percent alcohol for the same length of time. In a single experiment a 1 to 250 solution of nitric acid had little effect on the virus in 30 minutes, but a dilution of 1 to 100 destroyed it in one experiment but not in another. Bean virus 1, according to Pierce (*14*), was inactivated by a 1 to 200 dilution of nitric acid but not by a dilution of 1 to 500.

TABLE 5.—*Effect of chemicals on bean mosaic virus 4, as determined by production of local lesions on the Ideal Market variety*

Chemical	Concentration of chemical	Trial 1		Trial 2	
		Leaves inoculated	Total lesions	Leaves inoculated	Total lesions
		Number	*Number*	*Number*	*Number*
None		20	4,000–5,000	20	4,000–5,000
Alcohol	50 percent	20	3,000–4,000		
Do	75 percent	20	2,000–2,500	20	2,000–2,500
Do	95 percent			20	2,000–2,500
Nitric acid	1 to 250	20	4,000–5,000		
Do	1 to 100	20	300–400	20	0
Do	1 to 50	22	2	20	0
Do	1 to 25			20	0
Formaldehyde	1 to 1,000	20	4,000–5,000	20	4,000–5,000
Do	1 to 500			20	2,000–2,500
Do	1 to 200	20	2,000–3,000	20	2,000–2,500
Do	1 to 100	20	50–100	20	50–100
Sodium chloride	15 percent			20	0
Do	10 percent	20	50–100	20	0
Do	5 percent	20	100–150	20	50–100
Do	2.5 percent	20	1,500–2,000		

A 1 to 100 dilution of 37 percent formaldehyde did not completely inactivate bean mosaic virus 4. Bean virus 1, however, was inactivated by a 1 to 500 dilution (*14*, *28*).

A 30-minute exposure of bean mosaic virus 4 to a 15-percent solution of sodium chloride, which was then used as inoculum, injured the inoculated leaves so seriously that it was impossible to determine whether or not the virus was inactivated. In one test the virus was not inactivated in a 10-percent solution, although the inoculated leaves were seriously burned. In two tests (table 5) the virus was not inactivated in a 5-percent solution.

IMMUNOLOGICAL STUDIES

Price (*18*, *19*) showed that the relationship of certain viruses could be determined by means of cross-protection tests. Such tests were employed to determine the possible relationship between bean mosaic viruses 4 and 4A and bean virus 1.

Low Champion, a variety fairly susceptible to bean virus 1 and also to the local-lesion infections of bean mosaic viruses 4 (table 1) and 4A, was chosen for this study. Unlike the symptoms on many other susceptible varieties, those produced by bean virus 1 are quite distinct on the primary leaves when the virus is seed-borne. Since (table 1) it was noted that certain plants of this variety were resistant and others susceptible to local-lesion infection and systemic infection produced by bean mosaic viruses 4 and 4A, the immunological relationship could be determined for the two types of infected plants.

The lot of Low Champion used in this study carried a fairly high percentage of seed-borne bean virus 1. Twelve plants with mottled primary leaves infected with bean virus 1 were inoculated with bean mosaic virus 4, and 8 of these manifested local lesions. Eleven plants showing the symptoms of bean virus 1 were inoculated with bean mosaic virus 4A, and 7 manifested local lesions. Twenty-three healthy plants of the same variety were inoculated with bean mosaic virus 4, and 8 manifested local lesions. Eighteen healthy plants were inoculated with the bean mosaic virus 4A, and 12 of these exhibited local lesions.

It was shown earlier (table 1) that those plants of Low Champion that did not exhibit local lesions when inoculated with bean mosaic viruses 4 and 4A were always susceptible to the systemic infection. In order to determine whether the plants infected with bean virus 1 but not exhibiting local lesions when inoculated with bean mosaic viruses 4 and 4A actually became infected with these viruses or were immunized, further tests were made. Since the symptoms caused by bean virus 1 are not very different from the systemic symptoms produced by bean mosaic viruses 4 and 4A, it is impossible to determine whether viruses other than bean virus 1 are involved, except by inoculation.

Ideal Market was chosen to determine the presence or absence of bean mosaic viruses 4 and 4A in the plants mentioned above. An extract from each plant of Low Champion infected with bean virus 1 and later inoculated with bean mosaic virus 4 but not producing local lesions on Low Champion produced abundant local lesions on Ideal Market, indicating the presence of the virus. Extracts from the trifoliate leaves of plants whose simple leaves manifested local lesions did not produce local lesions on Ideal Market, proving the absence of systemic infection. This evidence, together with the fact that local lesions were produced by bean mosaic virus 4 on leaves infected with bean virus 1, suggests that bean virus 1 does not protect against bean mosaic virus 4; hence no apparent relationship exists between them. Identical results were obtained with bean mosaic virus 4A; hence it appears that no relationship exists between it and bean virus 1.

SEPARATION OF THE COMMON VIRUSES AFFECTING BEAN

The identification of bean virus 1, bean virus 2, bean mosaic virus 4, and bean mosaic virus 4A on the basis of symptomatology would be difficult, if not impossible, on certain varieties under field conditions. Under controlled conditions and on specific varieties, differentiation on this basis could be readily accomplished except with bean mosaic viruses 4 and 4A, provided each virus was pure.

Bean virus 1 is very similar in physical properties to bean virus 2 and, since it is quite sensitive to certain treatments, it cannot be separated from the other three viruses on the basis of those properties. It is seed-borne while bean virus 2 and bean mosaic virus 4 are not, and thus it can be separated from them by that means; it cannot thus be separated from bean mosaic virus 4A since the latter is also seed-borne. It can, however, be separated from bean mosaic viruses 4 and 4A by the inoculation of either of the strains of Pinto, since these viruses become localized in the inoculated leaves while bean virus 1 becomes systemic.

Bean virus 2 can be separated from bean virus 1, bean mosaic virus 4, and bean mosaic virus 4A by the inoculation of Corbett Refugee and Great Northern U. I. No. 59. Both varieties are resistant to bean virus 1 and also to systemic infection by bean mosaic viruses 4 and 4A. They are, however, susceptible to bean virus 2, which produces mottle symptoms on the trifoliate leaves.

Bean mosaic viruses 4 and 4A are readily separated from the other two by heating the mixture above 60° C. for 10 minutes. This temperature inactivates bean viruses 1 and 2. Similarly, diluting the mixture to 1 to 2,000 or above will inactivate bean viruses 1 and 2, but not bean mosaic viruses 4 and 4A.

DISCUSSION

From the data presented in this paper, it is evident that bean mosaic viruses 4 and 4A are distinctly different from any of the legume viruses thus far described on bean. Whether they are indigenous to bean or belong to another group of viruses is not known. It is believed that they probably belong to the legume virus group since, with the exception of *Phaseolus lunatus* and *Soja max* var. Virginia, they are largely restricted to *P. vulgaris*, and all bean varieties thus far tested are susceptible. Furthermore, bean mosaic virus 4A is seed-borne.

That other viruses produce local lesions on bean has been shown by Wingard (*23*) and Pierce (*14*) with tobacco ring spot virus, by Price (*17*) and Silberschmidt and Kramer (*20*) with tobacco mosaic virus, by Smith and Bald (*21*) with tobacco necrosis virus, by Zaumeyer and Wade (*27, 28*) and Pierce (*14*) with alfalfa mosaic virus, by Chamberlain (*5*) with pea streak virus (*Pisum* virus 3), and by Zaumeyer and Wade (*29*) with a virus from white clover (*Trifolium pratense* L.). None of these diseases shows any relationship to bean mosaic viruses 4 and 4A.

In table 6 are listed certain physical properties of most of the viruses that are infectious to bean. Although there is no apparent relationship between bean mosaic virus 4 and tobacco mosaic virus 1, certain of their properties, such as thermal inactivation and to some extent dilution, show some agreement. Pea streak virus is the only other one that shows some similarity to bean mosaic virus 4 in that it is inactivated at a very high dilution. On the other hand, its thermal inactivation point is considerably lower and it does not withstand aging as long in vitro.

It would probably be difficult definitely to separate or distinguish any of the above-mentioned viruses that produce local lesions on beans from bean mosaic viruses 4 and 4A on the basis of local lesions alone, with the exception of the pea streak virus of Chamberlain (*5*). Varietal differences aid in their separation. Likewise, the fact that bean mosaic viruses 4 and 4A produce local lesions on some varieties and systemic infection on others would differentiate them from tobacco mosaic virus 1, tobacco necrosis virus, and alfalfa mosaic virus and its strains, which produce no systemic infection in beans. In table 7 are listed a number of bean varieties showing their reaction to four viruses which produce local lesions on beans.

TABLE 6.—*Comparison of certain physical properties of bean mosaic virus 4 with those of other viruses infectious to bean*

Virus	Authority	Inactivation after indicated treatment		
		Temperature (10-minute treatment)	Dilution	Aging in vitro
		°C.		*Days*
Bean mosaic virus 4	Zaumeyer and Harter (*26*) and (present paper).	90–95	1 to 500,000	222
Bean virus 1	Pierce (*14*)	56–58	1 to 2,000	1–2
	Zaumeyer and Wade (*28*)	56–58	1 to 1,000	1–2
Bean virus 2	Pierce (*14*)	56–58	1 to 1,000	1–2
Pea virus 2	Osborn (*13*)	62–64	1 to 100,000	4–5
Pea streak virus (*Pisum* virus 3).	Chamberlain (*5*)	78–80	1 to 1,000,000	41
Pea mosaic virus 4	Zaumeyer (*25*)	62–65	1 to 10,000	1–2
Pea mosaic virus 5	do	60–62	1 to 10,000	1
Severe pea mosaic virus	Johnson and Jones (*11*)	60–70	1 to 100,000	15
Pea mottle virus	Johnson (*10*)	60–62	1 to 10,000	31
Pea wilt virus	do	58–60	1 to 100,000	31
Alfalfa mosaic viruses 1, 1A, and 1B.	Zaumeyer (*24*)	65–75	1 to 4,000	4–5
Alsike clover mosaic virus 1	Zaumeyer (*25*)	60–62	1 to 8,000	1–2
Alsike clover mosaic virus 2	do	54–58	1 to 10,000	1–2
White clover mosaic virus 1	Pierce (*15*); Zaumeyer and Wade (*28*).	58–60	1 to 3,000	5–7; 2–3
Cucumber mosaic virus strain 14.	Whipple and Walker (*22*)	65	1 to 10,000	8
Tobacco mosaic virus 1	Allard (*3*)	93	1 to 1,000,000	(1)
Tobacco ring spot virus	Pierce (*14*)	64–66	1 to 2,000	9
Tobacco necrosis virus	Smith and Bald (*21*)	72	1 to 10,000	8

[1] Indefinite.

The fact that bean mosaic viruses 4 and 4A produce either local or systemic infection on different bean varieties may possibly be explained on a genetic basis. A particular variety with a few exceptions (table 1) is either homozygous for resistance to the local lesions or homozygous for susceptibility to systemic infection, or vice versa.

It was originally assumed that the older leaves might carry the virus in a masked condition in certain varieties that exhibited the local lesions but no systemic infection. Many inoculations made from such plants to Ideal Market showed that no virus was present in the trifoliate leaves. Occasionally, owing to environmental or other unknown conditions, a series of inoculated plants of varieties known to be susceptible to the local lesions failed to exhibit them. It was thought that possibly when local lesions were produced they inhibited the spread of the viruses from those infection points, thus preventing systemic infection. Expressed juice from the trifoliate leaves of inoculated plants known to be susceptible to the local lesions but not exhibiting the symptoms, failed to produce local lesions upon inoculation to Ideal Market, proving that the virus was absent. Subjecting local-lesion-susceptible plants to high temperatures has never indicated the presence of the virus in any leaves above the inoculated ones.

Since bean mosaic virus 4A is seed-borne, it is assumed that it is more widespread than present information indicates. Because the symptoms on most varieties are not greatly unlike those produced by bean virus 1 and because the local lesions, if found under field conditions, would be very sparse and difficult to diagnose, it is believed that both viruses have been overlooked or confused with bean virus 1. Unless the juice from diseased plants is inoculated to varieties susceptible to local lesions or the properties of the virus in question are determined, such plants could be superficially classified as being infected with bean virus 1. It would be quite logical for any investigator in testing mosaic-infected beans to inoculate plants of varieties very susceptible to common bean mosaic, such as Stringless Green Refugee, most of which are resistant to the local lesions of bean mosaic viruses 4 and 4A. Unless a further study was made of these viruses, both of the new viruses could be readily overlooked. It is likely that this has been the case, and that actually they have been present in bean fields for several years.

TABLE 7.—*Comparative reactions of certain bean varieties to local lesions produced by bean mosaic virus 4, alfalfa mosaic virus 1, tobacco mosaic virus 1, and tobacco ring spot virus*

Variety	Reaction to—			
	Bean mosaic virus 4	Alfalfa mosaic virus 1	Tobacco mosaic virus 1	Tobacco ring spot virus
Blue Lake (White Creaseback)	+	...	+	+
Bountiful	−	+	−	+
Brittle Wax (Round Pod Kidney Wax)	−	+	−	+
Burpee Stringless Green Pod	−	+	−	+
Cutshort (Corn Hill)	+		+	...
Davis Stringless Wax	± [1]	+	−	+
Full Measure	−	+	+	+
Giant Stringless Green Pod	−	+	−	+
Horticultural, French	± [1]	+	−	+
Ideal Market	+		+	...
Improved Stringless Kidney Wax	−	+	−	+
Pencil Pod Black Wax	+ [1]	+	−	+
Red Kidney	−	+	−	+
Robust	−	+	+	+
Stringless Green Refugee	−	+	+	+
Striped Creaseback (Scotia)	+ [1]	...	+	...
Sure Crop Wax	−	+	−	+
Unrivalled Wax	−	+	+	+
Valentine, Black	−	+	−	+
Valentine, Red	−	+	−	+

[1] Heterozygous for susceptibility to local lesions.

Because of the susceptibility of most market garden varieties (table 1) to the systemic infection of bean mosaic viruses 4 and 4A, it is conceivable that these diseases may be more serious and destructive in the sections where these varieties are commonly grown than in most of the large territories devoted to growing canning beans. The Refugee types compose the principal varieties grown for canning purposes in some regions and, although nearly all show the systemic infection, they manifest mild symptoms when infected with bean mosaic viruses 4 and 4A. It is true, however, that in some canning sections, a high percentage of the acreage consists of extremely susceptible varieties such as Brittle Wax, Full Measure, and Asgrow Stringless Green Pod. It is possible that the actual damage resulting from these viruses will eventually cause a greater reduction in yield than the common bean mosaic, because the most generally grown varieties are the more susceptible to them.

It appears that bean mosaic viruses 4 and 4A are not as exacting in their temperature requirements for symptom expression as bean virus 1. The writers have observed that common bean mosaic is never as serious when the average temperatures are comparatively low as when the average temperatures are high. It is known, however, that at extremely high temperatures, such as are common during the summer months in most of the southern bean-growing States, bean mosaic symptoms may be masked.

Pierce and Hungerford (*16*) have shown that at temperatures between 18° and 21° C. a higher percentage of infection with bean virus 1 was observed than at lower temperatures. They likewise showed that 18 days were required for symptom expression at 21° as compared with 24 days at 8° to 9°. In addition, they noted severe dwarfing, curling, and mottling of the leaves at 26°, some curling and mottling at 22°, and only mottling at 18° to 19°. Fajardo (*6*) found that the mottle symptoms were partially masked at temperatures from 28° to 32°. Harrison's (*7*) results agree in general with those of Fajardo (*6*), who stated that the characteristic mottling was almost completely masked at 15° and at 30°, whereas from 20° to 25°, the mosaic mottling was distinct.

The studies reported in this paper showed that bean mosaic virus 4 required only 8 days for systemic symptom expression at 18°, 24°, and 27° C. and 13 days at 16°, whereas bean mosaic virus 4A expressed its symptoms in 5 days at 24°, in 7 days at 18° and 27°, and in 12 days at 16°. Distinct mottling was observed with both viruses at 16° and 27° after 20 days, at which temperatures the symptoms of bean virus 1 are partly masked.

Because of the shorter incubation period of bean mosaic viruses 4 and 4A and their less exacting environmental requirements for symptom expression, it is believed that the losses caused by these viruses throughout the country would probably be greater than those caused by bean virus 1 on the basis of equal distribution.

This might be particularly true in the Southern States where, according to numerous records in the Plant Disease Reporter issued by the United States Department of Agriculture, bean virus 1 is not as widespread and does not cause as much loss as it does in the Northern and Western States. The reasons for this may be (1) temperature relations and (2) the growing of fewer mosaic-susceptible varieties than in the North and West. Beans are generally not grown extensively in the South during the hot summer months, but principally during the seasons of the year when the average temperatures are not excessively high. Since bean mosaic viruses 4 and 4A produce distinct systemic symptoms at 16° and 18° C. and systemically infect many of the varieties that are commonly grown in the South, it is likely that these viruses may cause greater crop losses than bean virus 1.

It is of interest to note that only the sieva lima beans or beans closely related to them were susceptible to the local-lesion infections of bean mosaic viruses 4 and 4A. Harter (*8*) showed that lima bean mosaic virus (a strain of cucumber virus 1) reacted similarly, although this virus produced only a systemic mottle. No relationship exists between the lima bean virus and the two new bean viruses. These new viruses are also dissimilar to the two strains of cucumber mosaic described by Whipple and Walker (*22*), both of which systemically infected Henderson Bush Lima and one of them Fordhook Mammoth Pod.

Although all the 80 bean varieties of strains tested proved to be susceptible to either the local or systemic infection of both viruses (table 1), actually those varieties that do not express systemic infection can be considered commercially resistant. Even though local lesions caused by insect or mechanical dissemination may be found under field conditions, they would never be sufficiently numerous to cause defoliation such as is frequently noted under greenhouse conditions when plants are artifically inoculated. It is believed that this type of lesion would not be a factor in crop reduction.

In spite of the fact that bean mosaic viruses 4 and 4A appear to be closely related, there are some slight differences. On certain varieties of beans the local lesions produced by bean mosaic virus 4 are more diffuse and spreading (fig. 1, *A*, *B*) than those of bean mosaic virus 4A (fig. 1, *C*, *D*). In general, the systemic mottle produced by bean mosaic virus 4A on most bean varieties is more intense than that produced by bean mosaic virus 4. Bean mosaic virus 4A is carried in the seed as long as 7 months, whereas bean mosaic virus 4 is not seed-borne.

Some of the varieties resistant to bean virus 1, viz, Great Northern U. I. No. 59 and Corbett Refugee, are also resistant to the systemic infection of bean mosaic viruses 4 (table 1) and 4A. Idaho Refugee, also resistant to bean virus 1, is heterozygous for local and systemic infection; thus it is possible to select plants that are not systemically infected. U. S. No. 5 Refugee, Sensation Refugee Nos. 1066 and 1071, Medal Refugee, and Robust, all resistant to bean virus 1, are susceptible to systemic infection of bean mosaic viruses 4 and 4A. The symptoms produced on these varieties are mild under greenhouse conditions even though the virus is in a highly concentrated state.

The breeding of desirable canning, market, and field types for resistance to the systemic infection would not require a great deal of work, since there are a number of varieties of good type that are resistant to the systemic infection of the viruses and could be used as parental material. Furthermore, those varieties that are heterozygous for susceptibility to local lesions could be purified by propagating only from those plants that are susceptible to local lesions; these give rise to strains that are commercially resistant.

Since bean mosaic virus 4 is present in freshly ripened seeds but not in those that have been stored under laboratory conditions for at least 7 months, it is possible that the disease could be partly controlled by aging the seed for that length of time before planting. How long bean mosaic virus 4A would remain viable in dried seeds is unknown, but a period of 7 months did not destroy it completely.

SUMMARY

Two new closely related viruses of bean are described, identified, and compared with several other legume viruses and certain nonlegume viruses that are infectious to bean. The new viruses are designated as bean mosaic virus 4 and bean mosaic virus 4A. The exact distribution of these viruses is not known. Bean mosaic virus 4 has been isolated from beans growing in Louisiana, and bean mosaic virus 4A from California, Colorado, Idaho, and Maryland.

Bean mosaic viruses 4 and 4A produced local lesions on some bean varieties and systemic infection on others. No plant thus far tested has exhibited both types of symptoms, but some varieties produce some plants susceptible to one type and others susceptible to the other type.

The local lesions produced by both viruses are difficult to differentiate from those produced by several other viruses on beans. The systemic leaf symptoms closely resemble those caused by common bean mosaic (bean virus 1) on certain varieties but are different on others. On Stringless Green Refugee the symptoms of bean mosaic viruses 4 and 4A are very mild, whereas those caused by bean virus 1 are severe. On pods, bean virus 1 occasionally produces a mild mottling, while bean mosaic viruses 4 and 4A produce an intense mottling.

The susceptibility of 80 bean varieties or strains was determined. No variety was fully resistant to either of the 2 viruses. Twenty-four varieties were homozygous for susceptibility to the local-lesion infection of bean mosaic virus 4, 8 were heterozygous, and 48 resistant. The 48 resistant varieties were susceptible to systemic infection, and the 8 heterozygous were resistant. Thirty varieties were susceptible to the local-lesion infection of bean mosaic virus 4A, and 6 of these were heterozygous. Fifty varieties were resistant, all being susceptible to systemic infection. In general, the pole varieties were more susceptible to local infection than the bush varieties.

The sieva bean (*Phaseolus lunatus*) and closely related hybrids between *P. lunatus* and *P. lunatus macrocarpus* were susceptible to local infection of both viruses, but the Fordhook types were totally resistant.

The Virginia variety of *Soja max* was the only other suscept found among 31 species, representing 20 genera in 5 families.

Local lesions of both viruses were produced at temperatures ranging from 16° to 27° C. They appeared most rapidly at 27°. The systemic symptoms of bean mosaic virus 4 appeared in 8 days at temperatures ranging from 18° to 27°, but they were most severe after 20 days at 18°. The systemic symptoms of bean mosaic virus 4A were expressed most rapidly at 24° and were most severe after 20 days at 24° and 18°.

Both viruses were isolated from seed in the milk and dough stage and from freshly ripened seeds, but only bean mosaic virus 4A was isolated from seed stored in the laboratory for 7 months. Approximately 5 percent of such seed produced infected plants.

The two viruses were isolated from all portions of the systemically infected green plant. The lowest concentration was found in the roots.

The thermal inactivation point, resistance to aging in vitro, and tolerance to dilution were determined for the two viruses, and resistance to chemicals was determined for bean mosaic virus 4. Bean mosaic viruses 4 and 4A were inactivated between 90° and 95° C. They were still infectious at 1 to 500,000 dilution, and resisted aging in vitro at 18° for 32 weeks. Bean mosaic virus 4 was still infectious after being treated with 95 percent alcohol for 30 minutes. It was inactivated by a 1 to 100 nitric acid dilution in one experiment, but not by a 1 to 50 dilution in another. It was not destroyed by a 1 to 100 dilution of 37 percent formaldehyde for 30 minutes. Treatment for 30 minutes in a 5-percent solution of sodium chloride did not inactivate the virus. Immunological studies indicated no apparent relationship between the two new viruses and bean virus 1.

Bean mosaic viruses 4 and 4A can be separated from a mixture of bean viruses 1 and 2 by heating the mixture above 60° C. for 10 minutes or by diluting the extract above 1 to 2,000. The last two viruses are inactivated at these points.

The bean varieties that are susceptible to local-lesion infection can be considered commercially resistant. Such varieties are being used as parental material in breeding for resistance to systemic infection of other desired bean types.

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PHYSIOLOGICAL ASPECTS OF TETRAPLOIDY IN CABBAGE[1]

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INTRODUCTION

Comparisons of morphology and growth rates of colchicine-induced autotetraploids and their diploid progenitors have established the fact that, with few exceptions, autotetraploids are usually larger, coarser, slower in maturing, less fertile, and genetically more stable than the corresponding diploids. In genetically well-known plants, the morphological changes accompanying polyploidy can be rather precisely explained in terms of genic control and the doubling of factors governing size characteristics.

As to the internal, physiological consequences of polyploidy, we have only a meager and superficial knowledge from which no generalizations are possible at the present time. The value of additional information is obvious, and as more is learned concerning the physiological consequences of polyploidy, the morphological results of polyploidy may become less important by comparison, to the plant breeder.

The present paper is concerned with a comparison of some of the more important nutritional constituents of diploid and autotetraploid cabbage of similar genetic constitution (var. Ferry's Hollander).

HISTORICAL REVIEW

After a tentative study in which a biological test was used, Crane and Zilva (*2*)[2] suggested a correlation between chromosome numbers and antiscorbutic activity of diploid and triploid apples. Key (*4*) found no difference in the vitamin C content of diploid and tetraploid tomatoes, whereas Sansome and Zilva (*16*) reported the vitamin C potency of tetraploid tomatoes approximately twice that of the diploid. MacHenry and Graham (*8*) found no significant difference and questioned the validity of the work of Sansome and Zilva.

Kostoff and Axamitnaja (*6*) found little quantitative difference in the principal chemical compounds of normal and tetraploid petunias, but they noted marked differences between those of $2n$ and $4n$ tomatoes. The latter contained more water, nitrogen, proteins, and ash, but fewer carbohydrates than the diploids. Similar distinctions were found (*5*) in a comparison between F_1 hybrids and their amphidiploids.

The results of Kostoff and Axamitnaja were not substantiated by Fabergé (*3*), who found little difference in the materials produced by

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[2] Italic numbers in parentheses refer to Literature cited, p. 327.

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normal and tetraploid tomatoes. Tetraploid embryos were about 30 percent heavier, but this advantage was lost through their slower germination and the water content of the two types did not differ significantly. The reduction in the fruit weight of the 4*n* tomato has, of course, long been known.

Randolph and Hand (*14*, *15*) found an increase of 40 percent in the vitamin-A-potent carotenoid pigments of tetraploid corn as compared with its diploid progenitor, and Sullivan and Myers (*18*) found the colchicine-induced tetraploids of *Lolium perenne* to be higher in reducing sugars, sucrose, and total sugars than diploid plants of similar genetic constitution.

Straub (*17*) also reported a progressive increase of anthocyan and carotene content of a number of plants over a polyploid range from diploid to twelveploid in one instance.

In their studies on *Nicotiana*, Noguti et al. (*11*, *12*) reported that the nicotine content of autotetraploids of various species was 51 percent to 138 percent greater than in the corresponding diploids, and that the content of nitrates, sugars, resins, organic acids, totanitrogen, and ether extracts was also increased.

Another interesting physiological disturbance concomitant with tetraploidy in *Secale* has recently been reported by O'Mara (*13*). In *Secale*, a photoperiodic reaction, absent in diploid plants, was found in autotetraploids. This was responsible for the observed sterility. When adequate illumination was provided, fertility was restored, thus demonstrating that, in this case, sterility was due to egg or zygotic sterility and not to generational pollen sterility, as might have been suspected.

It is apparent from this brief summary of some of the investigations concerning the physiological consequences of polyploidy, that the evidence of the different workers is not in complete harmony nor have the investigations covered a representative group of economically important plants. Part of the lack of consonance may be explicable in terms of technique, but it seems also probable that the new genetic regime established by polyploidy might result in comparatively divergent physiological disturbances in even closely related plants.

EXPERIMENTAL METHODS

Tetraploid cabbage (*Brassica oleracea capitata* L.) produced at this station by one of the authors (*10*) was used as material for this investigation. At the outset, a thorough and extensive study of the chemistry of tetraploid cabbage tissue was designed, but curtailment of funds limited the investigation to some of the presumably more important substances, namely, ascorbic acid, carbohydrates, and nitrogen.

During the summer of 1941, plants were grown in the field until mature. They were then transplanted to 14-inch pots with a minimum of disturbance and kept in a coldframe for protection against low night temperatures until samples could be collected for chemical analysis. Some variation occurred in size of heads, but selections were made to obtain uniformity in size of the 2*n* and 4*n* heads for analysis.

Certain growth characteristics of the tetraploid plants are worthy of consideration here. The seeds were slower to germinate than those of diploid plants by about 3 to 6 days. The plants were correspondingly later in initiating head formation and the average weight of the mature heads was about 20 percent less than that of the diploid. The leaves were thicker and the petioles of the lower leaves were considerably larger and more spongy. In addition, the lower leaves of tetraploid plants tended to be more upright than those of the diploid plants which showed the normal tendency to spread and droop.

After the rest period of some of the diploid and tetraploid plants had been broken during the winter of 1941–42, the plants were grown in the greenhouse and allowed to flower. They were then selfed for the production of pure-line seed and the seeds were germinated. About 25 seedlings of each strain were transplanted and grown in the field during the summer of 1942.

SELECTION AND PREPARATION OF MATERIAL

In general, the procedure in sampling was as follows. After the head was selected, it was weighed, the outermost leaves were trimmed off, and it was then quartered on the axis parallel to the heart. One quarter was chopped to a convenient size and three 10-gm. samples were quickly weighed to ±.05 gm. and immediately placed in a mortar and ground to a paste with a pestle and clean quartz sand, in 3-percent metaphosphoric acid. These samples were used for the ascorbic acid determinations, details of which are given later. During this procedure another worker weighed duplicate 100-gm. representative samples which were preserved in boiling 95-percent alcohol for subsequent carbohydrate and nitrogen determinations. The time required to get the samples into the killing fluids (acid for the vitamin and alcohol for carbohydrates) was seldom more than 5 minutes. The samples for the ascorbic acid determinations usually were in the acid in less than 3 minutes.

DETERMINATION OF ASCORBIC ACID

The method used for the determination of ascorbic acid was that described by Bessey (*1*). The following modification was compared with the original procedure and found to be suitable and more convenient. Exactly 30 ml. of 3 percent metaphosphoric acid were added in 10-ml. portions to the tissue in the mortar. After the addition of the first 10-ml. portion of the acid the tissue was ground to a paste in the sand and acid mixture until all the cells had been ruptured to insure complete extraction of the vitamin by the acid. This has been shown (*1*) to be an important step, and satisfactory results may be obtained only with practice and after a number of preliminary tests have been run on the tissue under investigation. The remaining acid was then added with thorough mixing and the mass was transferred to a centrifuge tube and centrifuged for 10 minutes. A 5-ml. aliquot was then transferred to a 250-ml. beaker and diluted to about 70 ml., buffered to pH 3.5, and then transferred quantitatively to a 100-ml. volumetric flask and made to volume. A 4-ml. aliquot of this solution was then mixed with an equal volume of a standard 2,6-dichlorophenolindophenol solution in a photelometer cell and the milli grams of ascorbic acid calculated according to the equation given by Bessey (*1*), a fresh ascorbic acid solution being used as a reference standard. Except in certain cases the $2n$ and $4n$ samples were run parallel since fresh standard solutions of ascorbic acid and the dye were used at all times. This procedure eliminated the possibility of an error due to a change in reagent activity. In calculating the ascorbic acid in the sample a 90-percent water content of the tissue was assumed and taken into account.

It should be pointed out that the extraction of the ascorbic acid in the 1942 analysis was made by the use of a Waring blender and determinations made essentially as reported by Morell (*9*). A 50-gm. sample was weighed and placed in a blender cup containing 100 ml. of 3 percent metaphosphoric acid and blended for exactly 4 minutes. The mixture was filtered by suction and a 25-ml. aliquot was buffered to pH 3.5 and diluted to 100 ml. Four-ml. samples of this solution were then mixed with the dye and transmission readings made in a Cenco-Sheard-Sanford photelometer. Tests showed that this method of extraction was equally as effective in removing the ascorbic acid as by grinding with a mortar and pestle and quartz sand and was more convenient and timesaving.

The dye was standardized against carefully prepared solutions of ascorbic acid in 3 percent metaphosphoric acid buffered to pH 3.5. The calibration curves were obtained by plotting the log of the galvanometer readings against the various ascorbic acid concentrations in milligrams per 100 ml. of solution. The log of the galvanometer reading was log Gs—log Gsr, where Gs is the galvanometer reading of the sample plus the dye and Gsr is that of the sample with the dye completely reduced after the addition of a small crystal of ascorbic acid. Moderate turbidities are thus accounted for by the proper blanks with each aliquot sample from the plant tissue. The ascorbic acid in the aliquot is then obtained from the calibration curve and the amount per unit weight of the fresh plant tissue can be determined by a simple calculation.

DETERMINATION OF CARBOHYDRATES

The samples preserved in alcohol were subsequently extracted by decantation with boiling 80-percent alcohol. The extract was collected in 1,000-ml. volumetric flasks and made to volume. Preliminary tests showed that 11 to 13 decantations were necessary to completely remove the soluble carbohydrates, and 15 extractions were, therefore, used in order to insure complete extraction.

Two hundred milliliter aliquots of the extract were freed of alcohol, cleared with neutral lead acetate, and determinations were made for reducing and total sugars. Soluble nitrogen was determined on the alcoholic extract by the method described by Loomis and Shull (*7*).

No appreciable amino nitrogen was found in either the 2*n* or the 4*n* cabbage.

The residue remaining after the alcoholic extraction was used for starch and acid-hydrolyzable substance, colloidal nitrogen, and ash determinations.

EXPERIMENTAL RESULTS

Table 1 shows a summary of the results obtained for the constituents studied in the 2*n* and 4*n* tissue. Calculations were made on both the fresh-weight and dry-weight basis together with the percentage difference by the two methods of calculation.

TABLE 1.—*Chemical composition of diploid and tetraploid cabbage, 1941*

Constituent	Composition of normal diploid cabbage		Composition of tetraploid cabbage		Difference due to tetraploidy	
	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Reducing sugars	3.18	46.49	4.34	50.81	36.48	9.29
Total sugars	3.45	50.43	4.72	55.26	36.81	9.57
Starch	.86	12.57	1.30	15.22	51.16	21.08
Acid-hydrolyzable substance	.59	8.62	.84	9.83	42.37	14.04
Dry weight[1]		6.84		8.54		
Calculated dry weight[2]		5.96		7.65		
Ash content		4.94		3.99		—19.23
	Mg[3]	*Mg*[3]	*Mg*[3]	*Mg*[3]	*Mg*[3]	*Mg*[3]
Soluble nitrogen	204.6	2,991.22	175.80	2,058.54	—14.07	—31.18
Colloidal nitrogen	71.17	1,040.49	94.39	1,105.26	32.62	6.22
Ascorbic acid:						
Representative sample of head exclusive of outermost leaves and heart	48.40	707.60	59.95	701.99	23.86	—.79
Green outer leaves of mature plant	28.45	415.93	61.90	724.82	117.57	74.26
Heart[4]	94.30		110.40		17.07	
Lower green leaves of immature plant[5]	32.30	472.22	130.40	1,526.93	303.71	233.35

[1] Found by drying 100 gm. of fresh material to constant weight at 65° C. in a vacuum oven.

[2] Computed from the residual dry weights (dry weight after the 80 percent of alcohol-soluble materials were extracted) plus the calculated soluble substance estimated as sugars and soluble nitrogen.

[3] Expressed as milligrams per 100 gm. of plant tissue.

[4] These determinations were made on 10 gm. samples of heart tissue; no dry weight determinations were made on heart tissue.

[5] These samples were collected and analyzed on Sept. 3, 1941. The plants were immature with small heads. One of the lower leaves was removed from each of 12 different plants for the 2*n* and 4*n* samples; 4 composite samples were made of 3 leaves each from both 2*n* and 4*n*.

The data for the ascorbic acid values are averages of triplicate individual analyses from eight separate heads each of 2*n* and 4*n* cabbage. All other data are the averages of closely agreeing duplicate samples from the heads used for the ascorbic acid determinations. Statistical analysis of these data showed a significant difference at the 1-percent level in the ascorbic acid, sugars, and nitrogen fractions.

A comparison of the ascorbic acid content of 2*n* and 4*n* cabbage for 1941 and 1942, together with the range of variation found in the other varieties sampled, is shown in table 2.

TABLE 2.—*Ascorbic acid content of diploid and tetraploid cabbage*[1]

Cabbage	1941	1942	Range of other (diploid) varieties sampled
	Mg.	*Mg.*	*Mg.*
Diploid	48.40	53.51	40.20 to 57.00
Tetraploid	59.95	63.46	
Average			52.75

[1] Expressed as milligrams per 100 gm. of fresh tissue.

Ascorbic acid determinations on the 1942 crop agreed closely with results obtained in 1941. Results of ascorbic acid determinations on cabbage purchased on the open market varied from 40.20 to 57.00 mg. per 100 gm. of fresh tissue. The ascorbic acid content of two other varieties tested agreed closely also with that of the 2*n* plants grown for the experiment.

The ascorbic acid values for the determinations made in 1942 represent the average of 30 separate analyses from 15 heads of $4n$ cabbage and 56 analyses from 28 heads of $2n$ cabbage.

DISCUSSION OF RESULTS

It is apparent from the data that all the constituents studied in this investigation, with the exception of soluble nitrogen and ash, were higher in the $4n$ tissue than in the $2n$. Assuming the reducing sugars, the soluble nitrogen, colloidal or protein nitrogen, and ascorbic acid to be the most important factors from a nutritional standpoint, these substances would appear worthy of careful study.

The soluble carbohydrates found in cabbage tissue are chiefly reducing sugars. Small quantities of sucrose, by invertase hydrolysis, were found, but the amount present was less than 0.40 percent on the fresh weight basis in either the $2n$ or the $4n$ tissue. Although the sugar content of the cabbage plant is relatively low, tetraploid cabbage was found to contain 36.48 percent more reducing sugars than normal diploid cabbage.

The soluble nitrogen was less in the tetraploid tissue than in the diploid by about 14 percent, whereas the colloidal nitrogen was 32.62 percent higher in the tetraploid. The ascorbic acid content of the edible portion of the cabbage was 23.86 percent higher in the tetraploid than in the diploid. That of the green outer leaves from the diploid plants was moderately low, amounting to 32.30 mg. per 100 gm. of fresh tissue at the time when the heads started to form, and it did not exceed 28.45 mg. per 100 gm. at maturity. It is interesting to note in this connection that the lower leaves of immature tetraploid plants contained over 300 percent more ascorbic acid than comparable leaves of diploid plants. Later in the season, however, when the cabbage was harvested the ascorbic acid decreased about 3.85 mg. per 100 gm. in the diploid outer leaves while it decreased 68.50 mg. per 100 gm. in the tetraploid leaves. This behavior indicates that the formation of ascorbic acid in the mature head takes place without the accumulation of this substance in the photosynthetically active leaves.

Although the "heart"[3] tissue of tetraploid and diploid plants showed about the same percentage difference in ascorbic acid content as that found in representative samples of the head, there was a decidedly greater quantity of ascorbic acid in the heart than in the other parts of the head. In the tetraploid plants the ascorbic acid content was 84 percent greater in the heart tissue than in the part of the head commonly used for food. Diploid plants showed about 95 percent difference in the same tissues.

The surprisingly lower soluble nitrogen content of the tetraploid plants as compared with the diploid might be explained by assuming that during the development of the plant relatively more of the soluble organic nitrogen functioned in the synthesis of new $4n$ protoplasts, with the net result that more protein nitrogen was produced per unit of fresh weight. The information on the chemistry and physiology of polyploidy is so meager that we do not have a clear picture of the relations of the several internal factors, and it becomes, therefore, extremely dangerous to generalize. If, however, soluble organic nitrogen is associated with the synthesis of nucleoproteins and protoplasts it might be expected that the nonprotein soluble nitrogen would be lower in the tetraploid tissue since greater quantities of the simple nitrogen compounds may have been condensed into protoplasm and proteins in the production of cells and nuclei containing the greater number of chromosomes. Evidence to support this belief is the higher colloidal or protein nitrogen content of the tetraploid plants on both the fresh- and dry-weight basis in the present experiments.

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[3] The term "heart" as used here refers to the compact central part of the vegetable which is commonly not prepared for table use; the pith, exclusive of the surrounding conducting tissue.

No attempt was made in this study to determine the inorganic constituents. It was found, however, that the ash was definitely lower in the tetraploid plants than in the diploid.

SUMMARY

The ascorbic acid, sugars, starch, acid-hydrolyzable substances, and soluble and colloidal nitrogen content of tetraploid cabbage have been determined and compared with those of diploid tissue.

Tetraploid cabbage contained 36.48 percent more sugar,. 23.86 percent more ascorbic acid, and 32.62 percent more colloidal nitrogen than diploid cabbage. The soluble nitrogen was higher in diploid plants by about 14 percent.

The lower leaves of immature tetraploid plants contained over 300 percent more ascorbic acid than comparable leaves of diploid plants. The ascobic acid content of the outer leaves of tetraploid plants decreased with maturity. The decrease was evident but less pronounced in diploid plants.

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MEASUREMENT OF THE RESISTANCE OF PEAS TO APHIDS[1]

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INTRODUCTION

Studies looking toward control of the pea aphid, *Macrosiphum* (*Illinoia*) *pisi* (Kalt.), through use of aphid-resistant pea varieties were begun at Wisconsin in 1930 (*7*).[3] Determination of the aphid population at successive intervals during the season on peas growing in the field showed that varieties differ in the number of aphids borne upon them, and that certain varieties are relatively resistant (*8*). These early findings were corroborated by Maltais in 1936 (*5*). Initial attempts to approach the problem from the breeding standpoint were abandoned, however, because the procedure then in use was limited to testing plants en masse and could not be adapted satisfactorily to the detection of resistance in small segregating populations or in individual plants. In addition, data secured by means of the field procedure were not always amenable to satisfactory statistical analysis because of the multiplicity of factors affecting aphid populations under field conditions. In many cases, aphid populations were decimated by storms or by aphid parasites, predators, and disease before sufficient data could be obtained to show the comparative resistance of peas under test. Because of these disadvantages, it became apparent that before much progress could be made in breeding for aphid resistance in peas, a more dependable testing procedure would have to be developed. It was recognized that the new procedure must be able to give a rapid test with small numbers of plants, and that the data must lend themselves to satisfactory statistical analysis.

In 1939 major emphasis was shifted from field to greenhouse investigations, where detailed studies of host-parasite relationships pointed the way toward development of an accurate testing procedure (*4*). The new procedure is primarily adapted for greenhouse use, but may be used, with modified equipment, under field conditions as well. The principal advantages of the new procedure are: (1) Rapidity, (2) accuracy under variable environal conditions, and (3) adaptibility to the requirements of the pea breeder. The new procedure for measuring aphid resistance is described in this paper and an account is given of some results obtained with it. Considerable attention is given to the statistical analysis of the data because of the introduction of special statistical procedures.

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[2] The writers acknowledge their indebtedness to J. E. Dudley and T. Bronson, of the U. S. Department of Agriculture, and to H. F. Wilson and C. E. Dieter, of the Department of Economic Entomology of this station, for helpful advice in the course of the investigation, and to Frieda S. Swed and Mary Ann Lee, of the Agricultural Statistical Service, for calculations and other assistance in connection with tables 2, 3, 4, and 8.

[3] Italic numbers in parentheses refer to Literature Cited, p. 387.

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BIOLOGICAL BASIS OF THE EXPERIMENTAL PROCEDURE

The term "resistance" in this paper denotes the inherent ability of the plant to ward off or resist insect attack through an inhibitive influence on the insect biology. Susceptibility is considered the opposite of resistance. Both terms, however, are relative, there being no clear-cut line between plants which are slightly resistant and those which are susceptible; i. e., plants in which no inhibitive influence on the insect biology can be detected. This paper is not concerned with "tolerance," which may be described as the ability of a plant to support its insect population. The fact that a vigorous plant growing under ideal conditions can support a larger insect population than a weak plant in a poor environment exemplifies tolerance. Such plants may be equally resistant or susceptible.

Growth of the pea plant involves a progressive unfolding and expansion of parts arising from the meristematic tissues of the apical bud. This growth habit results in a gradient within the plant with respect to age of tissues, the apical tissues being youngest, those of the basal parts oldest. Within individual plants, greenhouse studies[4] have indicated that the rate of aphid development and reproduction, and the length of life of the insect, are inversely proportional to the age of tissues upon which the insects are confined. The inhibiting influence of older plant tissues appears to be associated directly with added age, and is independent of the variety of peas upon which it occurs. Differences in resistance between plants can be detected by differentials in developmental rates, reproductive rates, or in longevity of aphids confined upon any region of the test plants, provided that age of tissues used is identical for all plants under test (*4*).[4] If the aphids are confined to apical plant tissues, differences in total plant age may be overlooked, provided the test is run before blossoming and fruiting occur. Physiological changes of an unknown character associated with reproduction in the plant tend to cause irregular variations in aphid behavior.

The feeding of the pea aphid is not characterized by acute stigmonose, the injury apparently being confined to the withdrawal of necessary plant juices. Individual aphids cause, therefore, but slight damage, severe injury appearing only after sustained feeding by relatively large numbers of the pest. Because of this host-parasite relationship, the ultimate amount of injury to pea plants is determined principally by the numbers of aphids present, the duration of the infestation, and the tolerance of the host, i. e., its ability to replenish lost plant juices. The superiority of resistant over susceptible plants is due principally to the fact that their aphid populations increase at a slower rate, the insects requiring a longer infestation period to reach damaging numbers. The principal factor retarding population increases on resistant plants is the inhibition of aphid reproduction. Because of this fact, the criterion of resistance used in this technique is the rate of aphid reproduction on the plant under test.

Since temperature influences aphid reproduction, it must be carefully considered in utilizing data on aphid reproductive rates. In general, the velocity of aphid reproduction increases proportionately with rise in temperature, beginning at the developmental threshold (about 43° F.) and reaching a sustained maximum at 70° to 75° F. At higher temperatures, reproduction may be even faster for a time, but is not maintained continuously at these levels. Daily fluctuations of greenhouse temperatures cause comparable fluctuations in daily numbers of progeny produced by aphids, even though the insects are confined upon plants of similar resistance or susceptibility. When aphids are confined upon plants that differ in resistance, the daily number of progeny produced upon each will vary with day-to-day temperatures, but proportionately fewer young will always be produced on the more resistant plants.

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[4] HARRINGTON, C. D. Unpublished data.

If a number of plants of varying resistance are individually infested with a single newly reproducing aphid from a uniform stock, progeny counts after 6 to 8 days will reveal the comparative resistance of the plants tested. The value secured for each plant, however, cannot be safely compared with values secured for plants tested in previous or subsequent trials unless allowances can be made for temperature differences. In a comprehensive testing program the number of plants to be evaluated may be too large to include in a single trial. The first method used of terminating an experiment after a given interval of time, regardless of temperature, was abandoned, therefore, in favor of a system in which temperature is also a factor in determining the duration of the trial. This led to the adoption of accumulated "degree-hours" as the basis for deciding when a given experiment was to be concluded. According to this procedure, a trial is terminated, not after a given interval of time has passed following the initial infestation, but when a given number of effective degree-hours have accumulated. With this system there is nearly an automatic compensation in time for trials run under fluctuating temperatures. When prevailing temperatures approach the developmental threshold, effective degree-hours accumulate more and more slowly, thus prolonging the trial period. As the temperatures rise, the trial period is proportionately shortened. Merriman's developmental threshold of 43° F. is used as the base line, temperatures above this point being considered as effective in promoting aphid reproduction.

Within the optimum testing range of 55° to 75° F., changes in velocity of aphid reproduction are closely proportional to changes in rate of accumulation of effective degree-hours. Because of this relationship the number of aphids produced upon plants of similar resistance would be expected to vary only slightly from trial to trial. In greenhouses not equipped with devices for automatic control of temperature, however, temperatures often fluctuate beyond the optimum testing range. When fluctuations below 55° occur, aphid reproduction takes place at a slightly greater rate than effective degree-hours accumulate, while at temperatures above 75° the opposite is true because of an inhibiting influence of high temperatures on aphid reproduction. When temperatures fluctuate outside the optimum testing range, the average temperature of the experiment calculated at its completion is useful in indicating the extent to which the rate of aphid reproduction has deviated from the rate of accumulation of effective degree-hours. The importance and use of average temperature values will be discussed in a later section.

Because of the nearly straight-line relationship between rates of aphid reproduction and rates of accumulation of effective degree-hours, it would be expected that in a given number of accumulated degree-hours newly reproducing aphids of uniform stock confined upon comparable tissues of plants varying in resistance would repeatedly produce numbers of progeny in direct proportion to the congeniality of the host plants. This is the biological basis of the new testing procedure.

MATERIALS

Thirty varieties of field, garden, and canning peas (*Pisum sativum*) and one variety of English broadbean (*Vicia faba*) were used during the development and standardization of the techinque. Of the pea varieties, Perfection, Alaska, Gradawax, Onward, Pride, and Wisconsin Penin were most frequently employed. The plants were grown in soil in 4-inch pots or in No. 2 enameled tin cans with the sand culture method.

The aphid stock originated from a single agamic apterous insect collected from alfalfa. Since the pea aphid reproduces parthenogenetically, the desendants of this individual are presumably genetically identical. The use of pure-line insects was adopted to avoid differences in vigor which might occur in a random aphid population because of possible genetic heterogeneity. The aphid stock was maintained on the susceptible Perfection variety, growing in 6-inch pots in soil, and covered with cylindrical screen cages (fig.1).

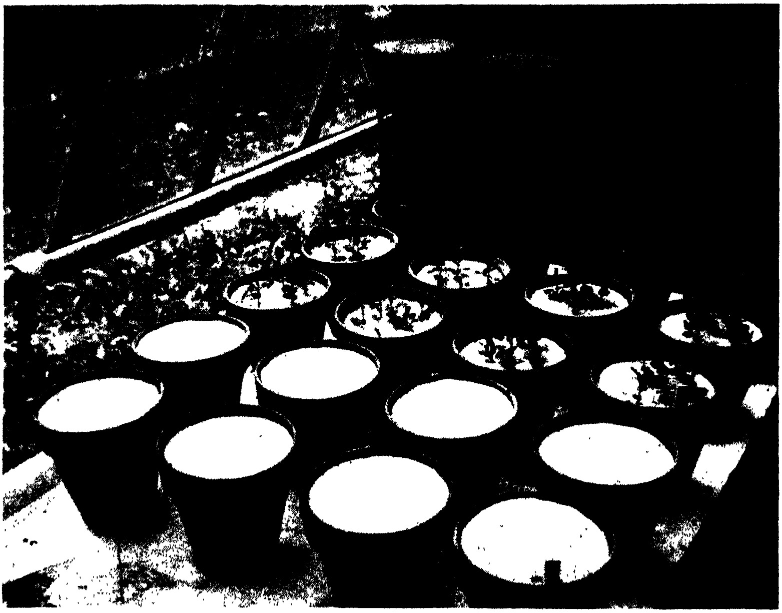

FIGURE 1.—Pots of Perfection peas planted at intervals to insure a continual supply of succulent plants for the aphid stock which is confined in cages at the rear. Note the sand layer over the soil to insure a tight fit between cage and pot

Mechanical equipment consisted of gauze-covered, clear-glass lantern globes for confining the aphids to the trial plants, and a series of racks for support of the plants and lantern globes. Ventilation was accomplished by directing the air stream from a number of 8-inch electric fans over the globes. Aphid progenies were removed from the plants with a mechanically powered aspirator constructed from a small electric vacuum cleaner. Temperature fluctuations during the progress of the trials were recorded by a thermograph. The arrangement of the equipment is shown in figure 2.

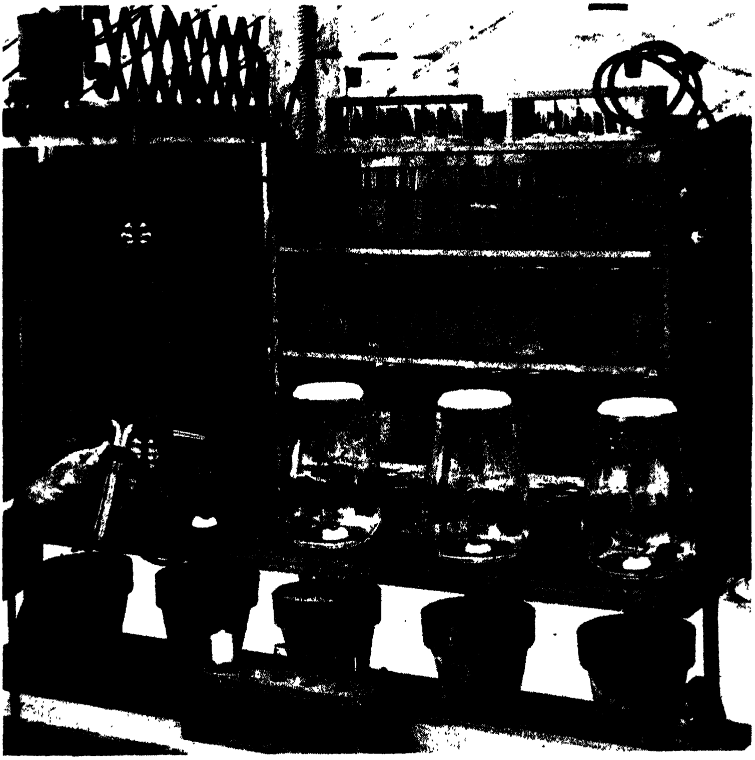

FIGURE 2.—Arrangement of testing equipment showing method of mounting plants in the testing racks, and the power-driven aspirator used to remove the aphid progenies from the plants at the close of the test. Note the detachable vial of the aspirator, and the filing system used to store the progenies until the counts could be made.

THE TESTING PROCEDURE

Plants to be tested are planted in individual containers and grown under as uniform conditions as possible. When the plants reach 4 to 5 inches in height (18 to 20 days after planting) the containers are mounted in the racks as shown in figure 2. A single freshly reproducing agamic apterous aphid is placed upon the tip of each plant with a camel's-hair brush, after which a lantern globe is set over the plant. The racks are placed in random order on a bench, the time is recorded, and the electric fans adjusted to insure ventilation of the globes (fig. 3).

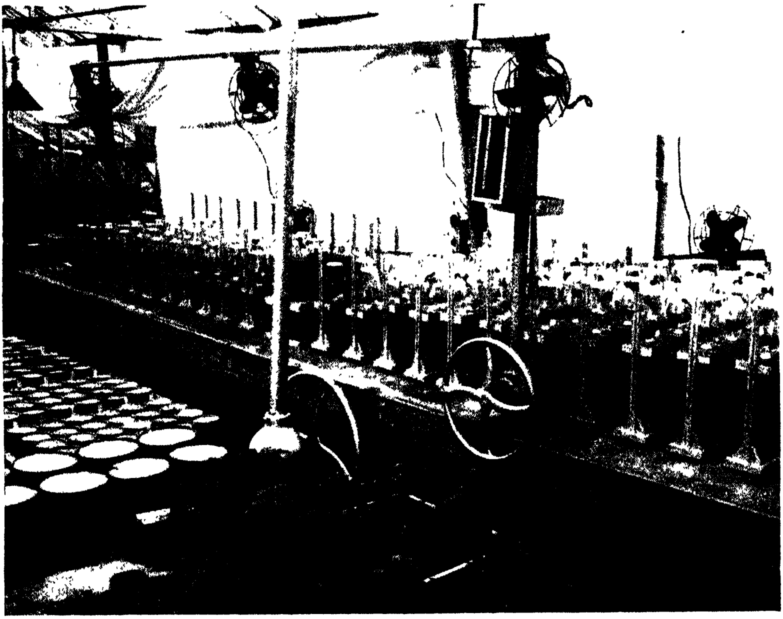

FIGURE 3.—Experimental set-up showing 22 testing racks used in a varietal test. Note the position of the thermograph and of the ventilation fans in the background. The fan rack in the foreground was raised in order to obtain a clearer view.

The aphids used in infesting the test plants are obtained from stock pots infested 8 to 9 days previously with 20 to 25 reproducing aphids. The parent aphids are allowed to reproduce on the plants only 4 hours, after which they are removed. The progeny produced during this short period are relatively uniform in age and vigor, and are of like genetic constitution. These aphids are placed on the test plants upon reaching reproductive maturity. It should be pointed out, however, that the stock plants must be in good condition and not overcrowded if a uniformly vigorous stock of apterous aphids is to be obtained. The presence of alate aphids in the aphid stock indicates that the insects may have suffered from lack of sufficient food.

The trial is ended when 4,750 effective degree-hours have accumulated. This number was found to be most satisfactory because it permits the parent aphids to produce the greatest possible number of progeny, yet stops the trial before their first-born young begin to reproduce. The results thus represent the reproductive efforts of the parent aphids alone. At average temperatures of 68° to 70° F., this number is reached on the eighth day.

At completion of the trial the aphids are removed from the plants with the power aspirator (fig. 2). The aphids on each plant are drawn into an individual vial which is then placed in a small vial rack numbered to correspond to the larger rack holding the test plant. After all progenies have been removed, the insects are counted. A few drops of chloroform, placed on a cotton plug and moved progressively from vial to vial before counting, anesthetizes the insects satisfactorily.

In addition to the number of progeny, other pertinent data are recorded. The condition of the parent aphid, whether normal, emaciated, diseased, parasitized, or dead, is noted. Normal parent aphids are usually found associated with susceptible host plants, while emaciated aphids, living or dead are usually associated with resistant plants. Low progeny counts resulting from disease or parasitism of parent aphids cannot be differentiated from low counts due to resistance without examination of the parent aphids. Disease or parasitism is rare, however, in adequately managed stocks. The number of progeny reaching the fifth instar before conclusion of the trial is also recorded. The presence of more than 10 of these insects per plant is a warning that second-generation offspring may have been produced. Such an occurrence is unlikely, however, unless the trial period is unduly prolonged.

The testing procedure will give fairly consistent results under widely fluctuating greenhouse temperatures (45° to 90° F.) provided, however, that the average temperature lies between 60° and 75° F. Average temperatures below 60° tend to inhibit reproduction on all plants to such an extent that small differences in resistance are masked. Average temperatures above 75° result in undue magnification of differences in resistance because of increased mortality of parent aphids on the more resistant plants. Although satisfactory results may be obtained between mean temperatures of 60° to 75°, 65° to 70° is the preferable range.

PRELIMINARY TRIALS OF APHID RESISTANCE IN PEAS

Three preliminary trials were made in consecutive order to determine the comparative resistance of six pea varieties—Perfection, Onward, Alaksa, Wisconsin Penin, Gradawax, and Pride. No attempt was made to stop these trials at the accumulation of 4,750 degree-hours, since this number had not been definitely fixed at that time. Trial 1 ran 4,390 accumulated degree-hours at an average temperature of 68.5° F.; trial 2, 5,008 at 68.8°; and trial 3, 4,186 at 67.3°. The results are presented in table 1.

The data in table 1 indicate that the six pea varieties vary considerably in congeniality to the pea aphid. These differences are expressed in each trial by variations in (1) mortality of parent aphids, (2) mortality of progeny, (3) numbers of progeny reaching fifth instar, and (4) average total numbers of progeny produced. It would appear that Perfection is most susceptible and that Alaska is more resistant than Perfection but less resistant than the other four varieties. Increased numbers of progeny on Perfection, Alaska, and Wisconsin Penin in trial 2 as compared with trials 1 and 3, together with increased numbers of progeny reaching the fifth instar, indicate that second-generation reproduction occurred during the additional time this trial was permitted to run.

TABLE 1.—*Aphid reaction on 6 pea varieties*

est lo.	Variety	Average parent mortality	Average progeny mortality	Average number of fifth instars	Average total progeny
1	Perfection	0	0	9.4	68.6
	Alaska	1	.6	8.4	50.7
	Wisconsin Penin	3	1.0	5.3	33.1
	Gradawax	7	2.5	4.0	29.4
	Pride	7	1.3	1.5	27.6
	Onward	6	1.2	1.3	24.2
2	Perfection	0	0	16.5	[1] 91.5
	Alaska	1	0	16.3	[1] 77.3
	Wisconsin Penin	6	.3	11.0	[1] 45.2
	Gradawax	6	.8	5.3	40.8
	Pride	8	1.3	7.4	33.7
	Onward	6	1.2	9.8	35.6
3	Perfection	0		7.0	61.3
	Alaska	2		6.6	50.7
	Wisconsin Penin [2]	7		5.3	37.9
	Gradawax	6		3.2	38.4
	Pride	4		3.3	39.3
	Onward	9		2.9	36.9

[1] Second-generation reproduction noted.
[2] Counts based on 9 plants, all others on 10 plants.

STATISTICAL TREATMENT OF THE DATA

Since the mathematical theory behind the usual analysis of variance tests of significance presupposes the existence of a common variance appropriate to all observations involved in the analysis, it was deemed well to proceed by first examining within each of the separate varietal trials the tenability of the assumption of a common within-variety variance. For this purpose, the Bartlett homogeneity test (*1*) was used with the results shown in table 2.

TABLE 2.—χ^2 *test of homogeneity of within-variety variances within trials*

Test No.	Degrees of freedom	χ^2
1	5	7.950
2	5	7.548
3	5	9.724
Total	15	25.222

Since the 5-percent significance level of χ^2 for 5 degrees of freedom is 11.07, no one of the observed χ^2's considered alone is statistically significant at this level. On the other hand, their sum, 25.222, is borderline—the 5-percent level for 15 degrees of freedom is 24.996—and throws suspicion on the assumption of a common within-variety variance. An examination of the variance estimates themselves did not reveal any variety or group of varieties which differed *consistently* in this respect from the others. Consequently, it was felt that the usual analysis of variance procedure could be applied without appreciable error to the data of a single varietal test.

Since a composite analysis of variance with data from all three varietal trials would require homogeneity of within-variety variance, not only within trials but also over all three trials, it was decided to find whether the three estimates of within-variety variance for each variety were homogenous. The results are presented in table 3.

TABLE 3.—χ^2 *test of homogeneity of within-variety variances for each variety*

Variety	Over-all trials		Trial 2 versus trials 1 and 3		Trial 1 versus trial 3	
	Degrees of freedom	χ^2 [1]	Degrees of freedom	χ^2 [1]	Degrees of freedom	χ^2 [1]
Perfection	2	8.166*	1	5.750*	1	2.402
Alaska	2	6.544*	1	6.007*	1	.533
Gradawax	2	.673	1	.371	1	.300
Pride	2	.263	1	.000	1	.261
Onward	2	5.025	1	.002	1	4.994*
Wisconsin Penin	2	9.506*	1	7.396*	1	2.097

*The values shown are the adjusted χ^2 values, the adjustment factor not being the same in all 3 columns.

[1] Significant at the 5-percent level.

From table 3 it is evident that the within-variety variances for Perfection, Alaska, and Wisconsin Penin are not homogeneous over the three trials, and that it is the value for trial 2 which is out of line in each case. The analysis has here detected the distortion caused by second-generation reproduction on these varieties in trial 2. If trial 2 data are omitted from the analysis, it appears that the assumption of homogeneity of trials 1 and 3 of the within-variety variance will be acceptable in all cases, except possibly in the case of Onward where early death of parent aphids unduly increased its internal variability.

With these points in mind, a composite analysis of variance was performed, using the data of trials 1 and 3. The results are presented in table 4.

TABLE 4.—*Analysis of variance, data of trials 1 and 3*

Variation	Degrees of freedom	Sum of squares	Mean square
Between varieties:			
Alaska and Perfection vs. others	1	15,983.50	15,983.50*
Alaska vs. Perfection	1	2,030.63	2,030.63*
Among others	3	240.18	80.06
Total	5	18,254.31	3,650.86*
Trial 1 vs. trial 3	1	820.90	820.90*
Varieties× trials:			
Alaska and Perfection vs. others	1	1,047.88	1,047.88*
Alaska vs. Perfection	1	199.83	199.83*
Among others	3	202.36	67.45
Total	5	1,450.07	290.01*
Within varieties	107	12,140.30	113.46
Grand total	118	32,665.58	

*Significant at the 5 percent level when the pooled within-varieties mean square, 113.46, is used to represent the experimental error. This composite mean square includes a portion from the Onward variety which appears to be a bit discordant. However, the exclusion from the analysis of this variety and its contribution to the estimate of "error" only changes the "error" to 114.82, which is a negligible change so far as the present analysis of variance is concerned. Therefore, for completeness, the Onward variety and its contribution to the estimate of "error" were retained.

It will be noted that the 5 degrees of freedom for "Between varieties" and for "Varieties×trials," have been resolved further into the comparison (a) of Alaska and Perfection with the other four varieties considered as a group, (b) of Alaska with Perfection, and (c) of the other four among themselves. This further decomposition leads to the following inferences on the basis of trials 1 and 3: (1) There are significant differences (at the 5-percent level) between varieties with regard to average aphid production; (2) the varieties Gradawax, Onward, Pride, and Wisconsin Penin do not differ significantly from each other in respect to average number of aphids produced, but do differ significantly from Perfection and Alaska, which in turn differ significantly in this regard. Trial 3 has the higher production.

VARIETAL TRIALS OF APHID RESISTANCE IN PEAS

The data in table 5 were secured from several similar trials of varietal resistance. Values given each variety represent the average number of young produced by 10 aphids confined individually upon 10 plants under the temperature-time conditions of its respective trial.

TABLE 5.—*Comparative aphid resistance of a number of pea varieties as determined by the use of the new testing procedure in 5 separate trials*

Varietal trial No.	Average temperature	Accumulated degree-hours	Variety	Average aphids
	° F.			*Number*
1	68.0	4,832	Triumph	88.1
			Early Wales	78.9
			Prince of Wales	77.8
			Profusion	76.3
			Asgrow No. 40	75.7
			Perfection (check)	72.8
			Asgrow No. 83	67.6
			Wisconsin Merit	65.5
			Surprise	62.5
			Yellow Admiral	55.7
2	71.1	4,793	Danby Stratagem	71.1
			Perfection (check)	68.7
			Dwarf Defiance	63.9
			Sherwood	60.2
			Potlatch	54.1
			Stratagem (regular)	44.0
3	72.2	4,675	Perfection (check)	73.1
			Horal	70.4
			Canner King	65.2
			Horsford	62.4
4	70.8	4,759	Fasciated Sweet	77.6
			Perfection (check)	72.7
			Emerald	62.9
			Confidence (dark)	60.3
			Glacier	59.3
			Confidence (light)	57.6
5	70.7	4,714	Perfection (check)	73.2
			Daniel	72.1
			Creole	68.4
			Cade	66.4
			English boardbean [1]	63.9

[1] *Viciafaba.*

THE COMPARISON EQUATION

Reference to the values obtained for the Perfection variety (which was the susceptible check) in the trials listed in table 5 shows that the values vary from trial to trial, although the number of accumulated degree-hours is approximately equal in all. This is because the trials were made under temperatures which frequently fluctuated above the upper limit of the optimum testing range, 55° to 75° F. When such fluctuations occur, the parallel relationship between rate of aphid reproduction and rate of accumulation of effective degree-hours is temporarily lost. Differences in the number and size of these fluctuations between trials caused the differences noted in the values obtained for the Perfection check. From this it is seen that the accumulation of a given number of effective degree-hours does not wholly determine the value obtained when a variety is tested under widely variable temperature conditions. The value is influenced to some extent also by the particular combinations of temperature and time which occur during the trial period. For example, a temperature of 70° F. acting for 4 hours will result in slightly more young aphids being produced than will temperatures of 50° and 73° F. acting for 6 and 2 hours respectively, even though the total number of accumulated degree-hours (108) is equal in both cases. The average temperature in the first instance is 70° while in the latter it is only 56.5°. The key to differences in number of aphids produced is obviously the average temperature. The total number of accumulated degree-hours is actually the sum of all temperature-time combinations occurring in a given period. The average temperature, on the other hand, is an expression of their average magnitude. The interrelation of average temperature and number of accumulated degree-hours is complex with respect to its influence on aphid reproduction. When a given variety is tested a number of times under varying conditions, it is found that the progenies produced in each trial are directly proportional to the products of their respective average temperatures and total number of accumulated degree-hours. This statement may be expressed algebraically by the following equation, hereafter called the "comparison equation":

$$\frac{V_1}{(ADH_1)\ (AT_1)} = \frac{V_2}{(ADH_2)\ (AT_2)}$$

where ADH_1 and ADH_2 denote the accumulated degree-hours and AT_1 and AT_2 denote the average temperatures for the first and second test, respectively, and V_1 and V_2 denote the corresponding expected numbers of progeny.[5] If this equation is valid, a ratio of approximately 1:1 should result when actual values are substituted and the equation simplified. Table 6, based on the data from trials 1 and 3 of the preliminary study and from the five trials of varieties, provides a series of empirical tests of the validity of the comparison equation. The values for V used in the equations are the values obtained for Perfection in the respective trials.

The terms of the ratios presented in table 6 are found to vary less than 5 percent in 18 of the 21 cases, the maximum difference found being 7.8 percent. This indicates that the reaction of the aphids to the Perfection variety is essentially the same when variations in time and temperature are thus taken into account.

The second-generation reproduction which occurred in trial 2 of the preliminary series prevented true estimates of resistance from being obtained for Perfection, Alaska, and Wisconsin Penin in this trial. If the value obtained for Perfection in this experiment is substituted in one side of the equation, table 7, the terms of the resulting ratios are found to vary considerably.

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[5] For a given set of time-temperature conditions there will correspond an expected number of progeny about which observed numbers of progeny will vary in successive trials as a result of sampling fluctuations.

TABLE 6.—*Values gained for Perfection under the differing conditions of 7 separate trials substituted in the comparison equation in all possible combinations to test the validity of the equation*

Combination No.	Perfection values, according to trial No., used in equation on—				Ratio
	Left side		Right side		
	Preliminary trial No.	Varietal trial No.	Preliminary trial No.	Varietal trial No.	
1	1	3	3		1.000=1.047
2	1	1		1	1.000=1.000
3	1	2		2	1.000=1.078
4		3		3	1.000=1.053
5		4		4	1.000=1.057
6		5		5	1.000=1.038
7	3			1	1.000=1.048
8	3			2	1.000=1.028
9	3			3	1.000=1.005
10	3			4	1.000=1.008
11	3			5	1.000=1.003
12		1		2	1.000=1.077
13		1		3	1.000=1.052
14		1		4	1.000=1.056
15		1		5	1.000=1.035
16		2		3	1.000=1.023
17		2		4	1.000=1.019
18		2		5	1.000=1.035
19		3		4	1.000=1.004
20		3		5	1.000=1.014
21		4		5	1.000=1.017

TABLE 7.—*Increased variation of the expected 1:1 ratio resulting when abnormal values due to second-geneartion reproduction are substituted in one side of the comparison equation*

Combination No.	Perfection values, according to trial No., substituted in equation on—			Ratio
	Left side	Right side		
		Preliminary trial No.	Varietal trial No.	
1	Preliminary trial No. 2	1		1.000=1.164
2		3		1.000=1.220
3			1	1.000=1.164
4			2	1.000=1.225
5			3	1.000=1.226
6			4	1.000=1.230
7			5	1.000=1.224

It is seen from table 7 that the differences between the two terms of the ratios vary from 16.4 to 23 percent. These computations show that the comparison equation may be used as a means of determining the comparability of data from separate trials. The more closely the resultant ratio approaches 1:1, the greater the degree of comparability between the trials. For practical purposes, two trials may be considered as comparable if the two terms of the ratio do not vary more than 10 percent (1.00=1.10), and the standard errors of the average values of V employed are not greater than 4 percent of these average values. If the standard errors are greater than this, averages based on larger numbers of observations should be employed.

COMBINING RESULTS OF SEPARATE TRIALS

After a number of varietal trials are completed, it may be desired to combine the results so as to show the relative resistance of all varieties tested on a single scale. Three methods of varying accuracy have been devised for this purpose. All are based, directly or indirectly on the comparison equation, and involve formulae whereby values obtained for the different varieties under the conditions of their respective trials are used to estimate the values which might be expected of these varieties under a common set of experimental conditions.

METHOD NO. 1, USE OF PERFECTION CHECKS

Within a given trial, all varieties are exposed to the same combinations of time and temperature, and from this the assumption can be made that any time-temperature variation which affects reproduction on the Perfection check variety will affect reproduction on other varieties to a proportionate degree. If the reaction of the aphids to the Perfection checks is found to be similar in a series of trials when variations of time and temperature are accounted for by the use of the comparison equation, then the values gained for Perfection can be used as a basis for determining the approximate resistance of all other varieties tested. For example, one may wish to know how Wisconsin Merit, tested in varietal trial 1, compares in aphid resistance to varieties tested in varietal trial 2. The following relation is used, PV_1 and PV_2 here denoting the average number of progeny on Perfection in the respective trials, MV_1 the average number of progeny on Wisconsin Merit in the first trial, and x standing for the estimate of MV_2, the number of progeny expected on Wisconsin Merit under the conditions of the second trial:

$$PV_1 : PV_2 = MV_1 : x$$

$$74.9 : 72.1 = 65.5 : x$$

$$74.9x = 47{,}226$$

$$x = 63.0$$

From this it is seen that Wisconsin Merit, which tested 65.5 in varietal trial 1, would be expected to test 63.0 under the conditions of varietal trial 2. This procedure, when applied to each variety of varietal trial 1, will result in a composite list showing the comparative resistance of all varieties included in the two trials. If results of a large number of trials are to be combined, estimated values of the different varieties may be computed by the above procedure for a set of experimental conditions not represented by any of the trials, provided, of course, that an estimated value of the Perfection check obtained by direct observation is known for this set of conditions.

METHOD 2, DIRECT USE OF THE COMPARISON EQUATION

If an estimated value of a variety obtained by direct observation is obtained for a particular set of conditions, use of the comparison equation will show the response which may be expected of this variety under any other set of experimental conditions. The formula used[5] is as follows:

$$\text{Estimated } V_2 = (\text{observed } V_1) \cdot \frac{(ADH_2)(AT_2)}{(ADH_1)(AT_1)}$$

Using the Wisconsin Merit value observed in variety trial 1 to calculate its response under the conditions of variety trial 2, the following result is obtained:

$$\text{Estimated } V_2 = 65.5 \frac{(4{,}793)(71.1)}{(4{,}832)(68.0)}$$

$$\text{Estimated } V_2 = \frac{22321221}{328{,}576} = 67.9$$

This procedure is more accurate than method 1 since it is based directly on the comparison equation, and does not involve the use of another variety. The reason for this will be discussed later.

METHOD 3, ESTIMATION BY INTERVAL

While an estimate of V_2 obtained by either of the above methods will generally be near the true value V_2, such an estimate gives no indication of its accuracy, and it is highly improbable that it will actually equal the true value V_2. In order to obtain an estimate with which there can be associated a chosen level of confidence, estimation by interval is necessary.[6] Thus, if an interval with a confidence coefficient of 0.95 is employed, the probability is 0.95 that the interval will include the true value within its range. Such an interval for log V_2 is given by

$$\text{estimated average } \log V_2 \pm t.05.s$$

where

$$\text{estimated average } \log V_2 = \log (ADH_2) + \log (AT_2) - \log (ADH_1) + \log (AT_1) + \text{observed average } \log V_1$$

$t.05$ is the 0.05 significance level of Student's t for $N-1$ degrees of freedom (*3*), and

$$s^2 = \frac{\sum(\log V_1)^2 - \frac{(\sum \log V_1)^2}{N_1}}{N_1(N_1-1)}$$

in which $\sum$ denotes summation over the N_1 values of log V_1 supplied by the first test. By taking antilogarithms the corresponding 0.95 confidence interval for V_2 is obtained.

As an example, the data for Perfection of trial 1 is used to predict the interval within which Perfection must lie for the standard conditions ($ADH = 4{,}750$; $AT = 68.0°$ F.):

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[6] NEYMAN, J. LECTURES AND CONFERENCES ON MATHEMATICAL STATISTICS. 160 pp., illus. [1938.] (U. S. Dept. Agr. Graduate School processed publication.) Washington, D. C.

Perfection V_1 values	Log V_1 values
75	1.875
68	1.832
65	1.813
62	1.792
65	1.813
61	1.785
78	1.892
68	1.832
76	1.881
68	1.832

$\log V_1 = 18.347$

$(\log V_1)^2 = 33.673549$

$ADH_1 = 4{,}390$

$AT_1 = 68.5$

$ADH_2 = 4{,}750$

$AT_2 = 68.0$

Estimated average $\log V_2 = 3.677 + 1.832 - 3.642 - 1.836 + 1.835 = 1.866$ Antilog $1.866 = 73.5$

$$s^2 = \frac{33.673549 - 33.661241}{(9)\ (10)} = .00013675$$

$s = .0117$ $t.05 = 2.262$ for 9 degrees of freedom

Interval $= 1.866 \pm 0.026$

$\log V_2$ ranges from 1.840 to 1.892

V_2 ranges from 69.2 to 78.0

Using method 3 in this case, an average value of 73.5 is predicted for V_2 and the probability is 19 in 20 that the true V_2 lies within the interval 69.2 to 78.0.[7] The V_2 value when predicted by the use of method 2 fell at 73.9 while the average V_2 value used as a basis for method 1 was 70.9, also within the interval. Thus it is seen that in this case the value predicted by the use of method 2 and that used in method 1 are consistent with the interval estimate of method 3.

If a 0.95 confidence interval is desired for V_1, it is given by observed average $\log V_1 \pm t_{.05} . s$, where $t_{.05}$ and s are the same values as in the preceding. Thus the 0.95 confidence interval for $\log V_1$ calculated from the above data is 1.835 ± 0.026; i. e. from 1.809 to 1.861, so that the 0.95 confidence interval for V_1 is 64.4—72.6.

STATISTICAL ANALYSIS OF COMPARISON EQUATION

The three methods described above for combining the results of a series of trials are based on the original comparison equation $V_1 : (ADH_1)(AT_1) = V_2 : (ADH_2)(AT_2)$. Since this equation occupies so important a place in analyzing the experimental data, it was decided to test its validity through statistical analysis of results obtained by its use. In order for this equation to be valid it is necessary that $\frac{V}{(ADH)\ (AT)}$ be equal to some constant which will depend on the variety concerned, over the range of time-temperature conditions likely to be met in practice.

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[7] It should be noted that the probability refers to the interval including the true value of V_2. It is *wrong* to say "the probability is 0.95 that the true V_2 will fall in the interval." The true V_2 is fixed (although unknown) when the variety and the time-temperature conditions are specified. It is the interval which varies as a result of sampling fluctuations, not V_2.

If $(AHD)(AT)/(V)=k$, a constant for a given variety, then taking logarithms, this implies that log $(ADH)+\log(AT)-\log\ (V)=\log\ k=K$, a constant independent of temperature conditions, also. Since this latter form is more convenient for statistical analysis, addition and subtraction, instead of multiplication and division, being easier to handle statistically, the analysis has been performed in terms of logarithms. From the data of preliminary trials 1 and 3 and variety trial 1, the values of log k for the Perfection variety, shown in table 8, were obtained.

TABLE 8.—*Values of log k for Perfection values from preliminary trials 1 and 3 and varietal trial 1*

Preliminary trial No. 1	Preliminary trial No. 3	Varietal trial No. 1
3.60	3.73	3.57
3.65	3.53	3.74
3.67	3.64	3.61
3.69	3.71	3.75
3.67	3.60	3.71
3.70	3.73	3.70
3.59	3.67	3.79
3.65	3.62	3.60
3.60	3.71	3.66
3.65	3.73	3.68
[1] 3.647	[1] 3.667	[1] 3.681

[1] Mean.

It can be seen that the three means differ in the second decimal place only. Using Student's t test, the comparisons P. T. 1 vs. P. T. 3, P. T. 1 vs. V. T. 1, and P. T. 3 vs. V. T. 1 yielded t values of 0.808, 1.327, and 0.449, respectively, all of which are seen to be less than the 5-percent significance level for 18 degrees of freedom (i. e., 2.101), indicating that there is no reason to suppose that log k, and therefore k, is different in the three tests. In like manner, the mean values of log k for preliminary trails 1 and 3 were compared for Gradawax and Alaska—these did not occur in variety trail 1—and the resulting values of t were 1.625 and 0.108, respectively, also nonsignificant. Therefore, it appears that, so far as a given variety is concerned, the ratio is sensibly constant; at least from the data at hand, no significant departure from constancy has been detected when the temperature conditions are varied within the range of temperature conditions of the data examined.

As regards precision, method 1 is definitely less precise than methods 2 and 3 since the error of estimation of MV_2, say, by method 1 involves the sampling errors of PV_1, PV_2, and MV_1, whereas the error of estimation of MV_2 by either method 2 or method 3 involves the sampling error of MV_1 only. Estimates obtained by method 1 are, however, sufficiently accurate for general purposes. Whereas method 1 cannot be employed unless data for a check variety, here Perfection, are at hand for both trial conditions, nevertheless, when it is applicable the simplicity of the calculation is a point in its favor.

Method 3 is the only one of the three which gives a clear indication—provided in this instance by the width of the confidence interval—of the accuracy of the estimate. When method 2 has been employed, a crude index of the accuracy of the estimate can be obtained without carrying out method 3 to completion by evaluating

Estimated geometric mean V_2=antilog (estimated average log V_2) and comparing this value with estimated average V_2 found by method 2. The estimated geometric mean V_2 will be less than the estimated average V_2 by an amount which depends on the variation among the observed V_2 values, equality being attained only when all of the observed V_2 values are equal. Thus, in table 9, it may be noted that method 2 gives 64.4 for Wisconsin Merit and for Cade and the corresponding geometric means are 54.6 and 64.1 respectively, indicating that the value for Cade is estimated more accurately than that for Wisconsin Merit. A glance at the relative widths of the corresponding confidence intervals obtained by method 3 bears out this inference.

TABLE 9.—*Comparative aphid resistance of pea varieties listed in tables 1 and 5 as calculated for standard trial conditions by the 3 prediction methods*

Variety	Method 1, average value	Method 2, average value	Geometric mean [1]	Method 3, interval
Onward	25.0	26.0	20.4	11.1 - 37.4
Pride	28.5	29.6	24.3	14.2 - 41.4
Gradawax	30.4	31.6	29.0	20.4 - 41.1
Wisconsin Penin	34.2	35.6	33.4	25.0 - 44.8
Stratagem	43.3	41.7	37.2	23.8 - 57.9
Potlatch	53.2	51.3	48.6	36.3 - 65.2
Yellow Admiral	52.7	54.8	51.2	38.6 - 68.1
Confidence (light)	56.2	55.2	53.2	43.2 - 65.6
Alaska	52.4	54.5	53.5	46.2 - 61.8
Wisconsin Merit	62.0	64.4	54.6	33.3 - 89.3
Glacier	57.8	56.8	55.3	46.6 - 65.8
Sherwood	59.2	57.1	56.2	49.7 - 63.7
Confidence (dark)	58.8	57.8	57.3	52.0 - 63.1
Horsford	60.5	59.7	59.7	54.6 - 64.4
Emerald	61.3	60.6	59.4	52.6 - 67.1
Surprise	59.2	61.4	59.4	49.0 - 72.1
Dwarf Defiance	62.8	60.6	60.0	54.6 - 65.9
Asgrow No. 83	64.0	66.5	60.5	42.5 - 86.3
English broadbean [2]	61.9	61.9	61.7	56.6 - 67.1
Canner King	63.2	62.4	62.2	58.9 - 65.8
Cade	64.3	64.4	64.1	59.4 - 69.2
Creole	66.3	66.3	66.2	64.0 - 69.2
Danby Stratagem	69.9	67.4	66.5	53.7 - 82.4
Perfection (variety trial 2)	[3] (70.9)	68.3	68.1	66.2 - 70.0
Perfection (variety trial 4)	(70.9)	69.7	69.5	66.1 - 73.1
Daniel	69.8	69.1	69.8	66.5 - 73.3
Perfection (variety trial 3)	(70.9)	70.0	69.8	66.2 - 73.6
Profusion	72.2	75.0	70.2	60.6 - 97.3
Perfection (variety trial 5)	(70.9)	71.0	70.8	66.4 - 75.5
Perfection (variety trial 1)	(70.9)	73.6	73.3	68.2 - 78.7
Perfection (preliminary trial 1)	(70.9)	73.9	73.5	69.2 - 78.0
Asgrow No. 40	71.7	74.4	73.6	65.8 - 82.4
Fasciated Sweet	75.7	74.9	74.0	69.8 - 78.3
Prince of Wales	73.6	76.5	75.9	68.6 - 84.0
Early Wales	74.7	77.6	77.3	69.7 - 85.7
Triumph	83.4	86.6	86.5	81.8 - 91.4

[1] Geometric mean = antilogarithm of average log V.
[2] *Vicia faba.*
[3] Estimated value.

It is recommended, therefore, that the method to be used be determined by the degree of accuracy desired. In a general testing program where a quick and easy method will suffice to show the relative resistance of varieties tested, methods 1 and 2 are sufficiently accurate. In technical studies where a certain degree of confidence is desired, method 3 should be used.

EXTENT OF APHID RESISTANCE IN PEAS

The known range of aphid resistance in peas is small. The most resistant pea varieties found to date are but midly resistant when compared, for example, with certain alfalfa lines (*2*), (*6*) which are almost immune to attack by the pea aphid. When compared with susceptible pea varieties, nevertheless, the difference is large enough to be of significance in the field.

Some 50 varieties of peas have been tested with the greenhouse technique. Three trials, comprising 18 varieties were discarded because of high temperature and consequent mortality of parent aphids. Varieties tested in preliminary trials 1 and 2 and in the 5 varietal trials were placed in their approximate positions in the resistance-susceptibility scale by means of the 3 methods described above. Values given the varieties in each of the 3 resultant scales are based on the probable response of these varieties under a standard set of experimental conditions, namely, 4,750 accumulated degree-hours at an average temperature of 68.0° F. No trials were run at exactly these standard conditions, so that no value was available for the Perfection check variety for these conditions. The value was determined, therefore, by averaging the hypothetical values predicted for the standard conditions by means of method 2 using the data from preliminary trial 1 and the 5 varietal trials. The value, 70.9, was obtained by this procedure. The results are presented in table 9.

Values predicted for the 31 varieties in table 9 by each of the three prediction methods are found to fall in approximately the same relative position, with respect to each other, in the resistance-susceptibility scale. All values obtained by the use of methods 1 and 2 fall within the interval necessary for 95 percent confidence as determined by the use of method 3. In fact, these values fall in the approximate center of the interval in the majority of instances. As noted above, the geometric mean predicted by method 3 will never exceed the corresponding arithmetic mean obtained by the use of methods 1 and 2. The size of the interval necessary for 95 percent confidence varies considerably from variety to variety depending upon the uniformity of progeny counts. In the case of Wisconsin Merit, Asgrow 83, and Profusion, the presence of some extremely low counts resulted in intervals much too large for practical application.

SUMMARY AND CONCLUSIONS

A new technique for detecting the presence and measuring the magnitude of aphid resistance in peas has been described. The principal advantages of the procedure are (1) accuracy under variable environal conditions, (2) rapidity, and (3) adaptability to requirements of the pea breeder. While the procedure is of value in supplying an efficient and rapid means of evaluating the comparative aphid resistance of established pea varieties and strains, its greatest promise lies in its ability to detect resistance in individual plants belonging to segregating families. The inability of pea breeders in the past to determine the comparative aphid resistance of single plant selections has been one of the principal factors in preventing the development of new pea varieties having increased resistance to the aphid. No variety of pea has yet been found that exhibits a high degree of resistance to the aphid, and for this reason it seems unlikely that new varieties of greatly superior resistance will be developed in the near future. Nevertheless, the incorporation of partial resistance, such as is found in the Onward variety, into other peas having more desirable canning qualities doubtless would be of real value to growers and packers of this staple crop.

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COMPARATIVE ABILITY OF SEVERAL SPECIES OF LYGUS AND THE SAY STINKBUG TO DAMAGE SUGAR BEETS GROWN FOR SEED [1]

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INTRODUCTION

In earlier work [2] the writer has shown that *Lygus hesperus* Knight and *L. oblineatus* (Say) and two of the stinkbugs—*Chlorochroa sayi* Stål and *Thyanta custator* (F.)—may render nonviable the seeds of sugar beets (*Beta vulgaris* L.) grown for seed. These two species of *Lygus* and also *L. elisus* Van Duzee occur in the seed-beet fields of the Southwestern States. The same species do not predominate in all districts, however, and field observations have indicated that all species may not cause equal damage. An investigation was therefore undertaken at Phoenix, Ariz., in 1940 to determine the potential as well as the comparative ability of the various forms of *L. hesperus*, *L. oblineatus*, and *L. elisus* to reduce the viability of seed balls of sugarbeets grown for seed, and to compare this damage with that caused by adults of *Chlorochroa sayi*.

MATERIALS AND METHODS

In December 1939 three 16-mesh screen cages measuring 10 by 15 by 7 feet were placed in a sugar-beet field over groups of plants to protect them from infestation by *Lygus* and other seed-feeding insects (fig. 1). At this time very few insects were present, but to insure insect-free conditions the plants within the cages were sprayed with a strong mixture of pyrethrum-in-oil.

The following April, after the plants had developed seedstalks, sleeve cages approximately 7 inches in diameter and 14 inches long were tied on individual spikelets of the plants within the large cages. These sleeve cages were made of curtain scrim having approximately 32 meshes to the inch, which is sufficiently fine to retain even small *Lygus* nymphs. The cages were set up in randomized blocks, 11 cages constituting a block, and each block was replicated 10 times. A sufficient number of flowers were included in each cage to develop from 300 to 400 seed balls, which was considered an excess of food for the insects. A single insect was then introduced into each cage so that each block would contain 1 female, 1 male, and 1 nymph of *L. hesperus*, *L. oblineatus*, *L. elisus*, and a *Chlorochroa sayi* adult without respect to sex; and 1 cage was maintained insect-free as a check. All the cages of 1 block were placed on the same beet plant to avoid any possible differences between plants. Figure 2 shows a close-up of 1 of the blocks within the screen cage.

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[1] Received for publication November 25, 1942.

[2] HILLS, O. A. ISOLATION-CAGE STUDIES OF CERTAIN HEMIPTEROUS AND HOMOPTEROUS INSECTS ON SUGAR BEETS GROWN FOR SEED. Jour. Econ. Ent. 34: 756–760, illus. 1941.

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Insects for this work were reared as pure-species colonies in cloth-covered cages measuring 4 by 5 by 7 feet, shown at the extreme left in figure 1. For the tests of nymphal damage nymphs were taken directly from these colony cages and placed in the sleeve cages. Nymphs as nearly as possible in the second instar were used and allowed to mature within the cage. The newly emerged adults were removed immediately and replaced with other second instars of the same species. For tests of adult damage individually reared specimens were used in order to prevent reproduction within the cages containing females. Adults were obtained by placing large nymphs from the colony cages in individual rearing cages on beet spikelets (fig. 3). As adults emerged, males and females were placed in separate sleeve cages. The insects were observed every 2 or 3 days, and dead individuals were replaced with live specimens.

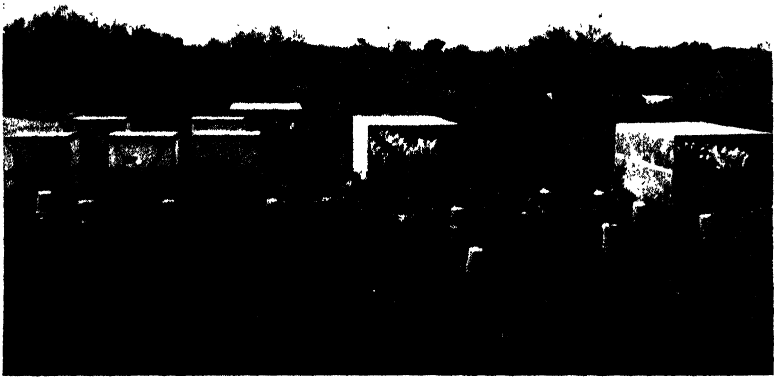

FIGURE 1.—Cages used in insect-isolation studies. The large cloth-covered cages at the left contained pure-species colonies. The three large screen cages were for protection of the plants from outside infestation; the small sleeve cages within contained the insects being tested. The cylindrical screen cages in the foreground were not used in this experiment.

The insects were maintained on the plants from the beginning of the blooming period until the seed matured, which in most cases was from May 10 to June 24. The average number of individuals used in maintaining one insect in each cage during this period was: For *Lygus hesperus,* females 1.4, males 2.1, and nymphs 3.9; for *L. oblineatus,* females 1.3, males 2.1, and nymphs 3.9; for *L. elisus,* females 1.4, males 1.9, and nymphs 4.9; for adults of *Chlorochroa sayi,* 1.4.

When the seed was mature, the sleeve cages were brought into the laboratory, where the seed balls [3] were stripped from the spikelets by hand and screened over a $\frac{7}{64}$-inch mesh hand screen to remove trash and small seed balls. The seed balls from each cage were then counted and germination analyses made to determine their viability.

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[3] In this paper the term "seed ball" is used to denote the dried fruit (diclesium) containing one or more true seeds, and is synonymous with the term "beet seed" in commercial usage.

Since it was desirable to know whether the insects were capable of feeding on one seed without damaging other seeds in the same ball, or whether they rendered the entire ball nonviable, counts were made not only of viable balls but also of the sprouts developing from each ball.

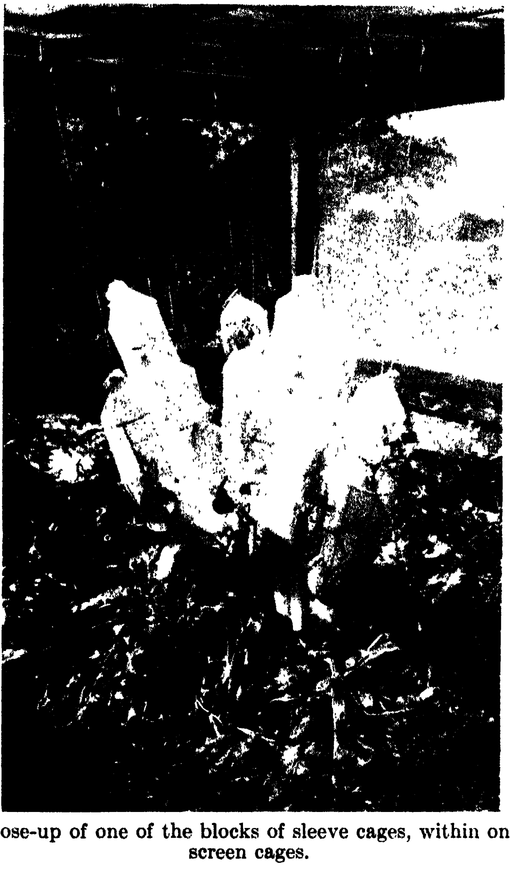

FIGURE 2.—Close-up of one of the blocks of sleeve cages, within one of the large screen cages.

RESULTS

The results of the tests are summarized in table 1. There was a significant increase in the number of nonviable seed balls in all cages containing insects over that in the check cages except in the cages containing *Lygus elisus* males. More nonviable seed balls were found in the *Chlorochroa sayi* cages than in any of the *Lygus* cages. There was a definite reduction in the number of sprouts per viable ball only in the case of damage by *C. sayi*. A slight reduction is indicated for *L. hesperus* nymphs, but in general feeding by *Lygus* spp. rendered all the seeds within the ball nonviable, whereas in cages containing *C. sayi* one or more of the seeds in certain balls were damaged but the other seeds within the ball germinated.

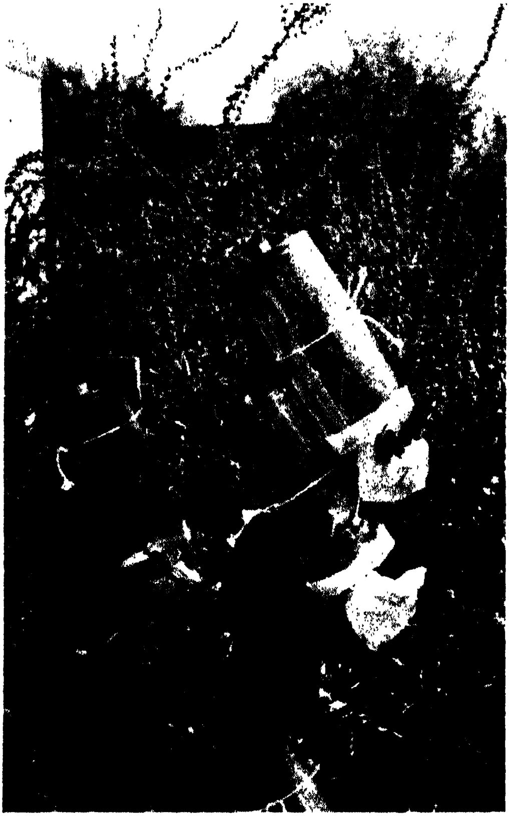

FIGURE 3.—Close-up of individual rearing cages, tied on tips of sugar-beet spikelets.

TABLE 1.—*Viability of seed balls from sugar-beet spikelets confined in cages with individuals of Lygus spp. and Chlorochroa* sayi; *means of 10 replicate cages*

Insect	Seed balls per cage			Sprouts per viable ball per cage
	Nonviable	Viable	Total	
	Number	*Number*	*Number*	*Number*
Lygus hesperus:				
Female	96.8	344.4	441.2	1.48
Male	62.8	370.3	433.1	1.53
Nymph	114.4	325.1	439.5	1.46
Lygus oblineatus:				
Female	96.7	325.5	422.2	1.56
Male	65.1	383.8	448.9	1.58
Nymph	79.0	324.5	403.5	1.61
Lygus elisus:				
Female	65.1	360.7	425.8	1.58
Male	54.0	355.2	409.2	1.56
Nymph	65.5	365.8	431.3	1.69
Chlorochroa sayi	206.1	126.7	332.8	1.27
None (check)	23.4	425.1	448.5	1.62
Difference required for significance	36.0	----------	(1)	.15

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[1] Not significant by the *F* test.

Since the cages were placed on the plants when they were in the very early blossom stage, it was impossible to encage a definite number of seed balls, and the only way to approach uniformity in this regard was to encage approximately the same length of each spikelet. However, the fact that there were no significant differences between treatments in total number of seed balls produced per cage indicates that none of these insects cause a reduction in the number of seed balls produced, which is in accordance with previous experiments.

Since the number of viable seed balls remaining in the *Lygus* cages was far in excess of the number of nonviable seed balls, it is probable that the damage caused by these insects had reached its maximum, and that no more nonviable seed balls would have been produced if larger numbers had been present. The number of viable seed balls remaining in the *Chlorochroa sayi* cages was considerably smaller than in the *Lygus* cages, and if these cages had contained more seed balls the number of nonviable balls might have been greater.

The mean differences between the number of nonviable seed balls occurring in cages containing the various forms and species of *Lygus* become more evident when the data pertaining to *Lygus* are analyzed without the data from the *C. sayi* or check cages. Table 2, in which the different forms of *Lygus* are grouped without respect to species, shows that the females and nymphs cause an equal amount of damage, but somewhat more than that caused by the males. Table 3, showing the same data for the three species of *Lygus* without respect to form, indicates that *Lygus elisus* damages a significantly smaller number of seed balls than does either *L. hesperus* or *L. oblineatus*.

TABLE 2.—*Viability of sugar-beet seed balls produced in cages containing males, females, and nymphs of Lygus without respect to species*

Form	Seed balls per cage	
	Nonviable	Viable
	Number	*Number*
Adult female	86.2	343.5
Adult male	60.6	369.8
Nymph	86.3	338.5
Difference required for significance	15.2	

TABLE 3.—*Viability of sugar-beet seed balls produced in cages containing various species of Lygus without respect to form*

Species	Seed balls per cage	
	Nonviable	Viable
	Number	*Number*
Lygus hesperus	91.3	346.6
Lygus oblineatus	80.3	344.6
Lygus elisus	61.5	360.6
Difference required for significance	15.2	

The results of these experiments not only furnish a basis for comparing the amount of damage to sugar-beet seed attributable to the various species and forms of *Lygus* as well as to adults of *Chlorochroa* *sayi*, but also, since they are based on the actual number of seed balls damaged, they give some idea of the damage to be expected from each individual occurring in the field. The fact that *Lygus* nymphs cause as much damage as the females is of prime importance, since at certain seasons the nymphs are far more numerous in the fields than the adults. The fact that *L. elisus* causes less damage than the other two species is also important in evaluating field populations of *Lygus*.

Less emphasis has been placed on *Chlorochroa sayi*, since this insect is usually present in beet fields in much smaller numbers than *Lygus*. In certain years, however, *C. sayi* has been known to occur in some areas in large numbers with disastrous results. This can easily be understood by the results of this experiment, which show that a single specimen maintained on the plant during the fruiting period is capable of destroying many more seed balls than any of the species of *Lygus*.

SUMMARY

This paper reports the results of a study to determine the extent of damage that may be caused to sugar beets grown for seed by several forms of three species of *Lygus* that occur in the seed-beet fields of the Southwestern States and by adults of *Chlorochroa sayi* Stål. The experiments were conducted with insects caged individually on seed-beet plants grown within large screen cages. *L. hesperus* and *L. oblineatus* caused more damage than *L. elisus*. Nymphs caused as much damage as females, and both nymphs and females caused more damage than males. Adults of *C. sayi* caused more damage than any of the species or forms of *Lygus*.

INFLUENCE OF VARIOUS FACTORS ON THE STARCH CONTENT OF KANSAS-GROWN POTATOES AND SWEETPOTATOES [1]

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INTRODUCTION

In addition to its value as a food material, the potato (*Solanum tuberosum* L.) has achieved considerable commercial importance because of the desirability of its starch for use in the textile industry. Recent studies (*8*)[2] have indicated that starch from sweetpotato (*Ipomoca batatas* (L.) Lam.) may also be of industrial value and an experimental mill has been in operation at Laurel, Miss., since 1934.

Systematic studies (*4, 6, 9, 10, 11*) of the starch content of both the potato and the sweetpotato have been made for a number of localities. These studies have revealed a rather wide variation in starch content. The results of a similar study of Kansas-grown potatoes and sweetpotatoes are reported in the present paper. For this work a series of carefully selected, representative samples was obtained. Since it was known that starch content may be influenced by a number of factors, the samples were selected in such a manner that as many of these factors as possible might be studied. However, the choice of samples was limited by the fact that they were collected by one man in the course of his regular duties.

The following factors were believed to affect the starch content of potatoes: Variety, stage of maturity, soil, previous crop grown on the land, and storage. Subsequent paragraphs will indicate the way in which these factors were taken into consideration during the course of the work.

The Irish Cobbler, Warba, and Bliss Triumph potatoes were selected because they are the varieties most commonly grown in Kansas.

The regular harvest season for Kansas in 1939 was July 11–14. Samples taken from the ground prior to July 11 were immature and were labeled "early harvest." Samples taken 5 to 10 days after the regular harvest had been completed were designated "late harvest." No record of the previous crop was obtained except in those instances specifically mentioned.

Two identical samples of Irish Cobbler were used to study the effect of storage. One sample was placed in cold storage (4.4° C.) and the other was stored in a shed. Weight changes as well as starch content were determined for each sample at monthly intervals for a 6-month period.

For sweetpotatoes the following factors were studied: Variety, locality, stage of maturity, and curing. The Little Stem Jersey, Big Stem Jersey, Improved Big Stem Jersey, Red Bermuda, and Nancy

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[1] Received for publication November 5, 1942. Contribution No. 262 of the Department of Chemistry, Kansas Agricultural Experiment Station.

[2] Italic numbers in parentheses refer to Literature Cited, p. 406.

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Hall were selected as varieties common to Kansas. Samples taken before October 1 were regarded as "early harvest"; those taken after that date were considered as "regular harvest." Five samples of regular harvest potatoes were put through the curing process which consisted of heating in a kiln at 26.7° to 32.2° C. for 10 to 14 days.

EXPERIMENTAL MATERIALS AND METHODS

COLLECTION OF SAMPLES

Thirty-six samples—18 of potatoes and 18 of sweetpotatoes—were collected during the period July to November 1939.[3] They were shipped to Manhattan, Kans., where they were placed in storage, the potatoes at 4.4° C. and the sweetpotatoes at room temperature, until used for the preparation of the analytical samples or the extraction of starch. Not more than 60 hours (average about 36) elapsed between the digging of a sample and the time it reached storage. The analytical samples were prepared in less than 24 hours after the samples were received; starch was removed from corresponding samples soon thereafter. A detailed description of the samples is given in tables 1 and 2.

TABLE 1.—*Data on field samples of Kansas-grown potatoes collected for analysis as to starch content*

Sample No.	Variety	Source	Type of soil	Stage of harvest	Starch (fresh weight basis) obtained when analyzed by—	
					Acid hydrolysis	Diastase hydrolysis
					Percent	*Percent*
1	Irish Cobbler	Near Edwardsville	Sandy loam	Early	[1] 13.26	11.77
4	do [2]	Newman Experimental Fields, Newman.	Fine sandy loam	do	14.67	13.40
5	do [3, 4]	do	do	Regular	14.31	12.55
8	do [2]	do	do	do	15.41	13.84
9	do [5]	do	do	do	14.64	13.22
10	do [2]	Near Loring	Loamy sand	do	13.28	11.86
11	do [6, 7]	Near Manhattan	Sandy loam	do	14.67	13.28
12	do [7, 8]	do	do	do	14.67	13.28
13	do	Near Edwardsville	do	Late	12.18	10.99
16	do	Newman Experimental Fields, Newman.	Fine sandy loam	do	13.30	11.98
17	do	Near Newton	Loamy sand	do	12.46	11.15
18	do [2]	Near Atchison	Upland	do	15.19	13.25
2	Bliss Triumph	Near Linwood	Sandy loam	Early	10.99	9.90
6	do [3, 4]	Newman Experimental Fields, Newman.	Fine sandy loam	Regular	11.45	9.90
15	do	Near Linwood	Sandy loam	Late	9.81	8.80
3	Warba	Near Edwardsville	do	Early	12.59	11.17
7	do [3, 4]	Newman Experimental Fields, Newman.	Fine sandy loam	Regular	13.56	11.90
14	do	Near Edwardsville	Sandy loam	Late	11.82	10.51

[1] Because of unsatisfactory handling, this sample will be disregarded in the discussion.
[2] From land on which potatoes were grown the previous year.
[3] Used in variety comparison.
[4] From land on which oats (followed by sweetclover, which was a failure) was grown the previous year.
[5] From land on which alfalfa was grown the previous year.
[6] Used in cold-storage (4.4° C.) experiment.
[7] Graded: Grade 2.
[8] Used in shed-storage experiment.

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[3] The samples were collected by Dr. O. H. Elmer of the Department of Botany, Kansas State College, in connection with his field work among the potatoe growers of the State.

TABLE 2.—*Data on field samples of Kansas-grown sweetpotatoes collected for analysis as to starch content*

Sample No.	Variety	Source	Stage of harvest	Starch (fresh-weight basis) obtained when analyzed by—	
				Acid hydrolysis	Diastase hydrolysis
				Percent	*Percent*
1	Little Stem Jersey	Kansas River Valley	Early	15.21	13.44
4	do	do	Regular	16.88	15.13
5	do	do	do	16.57	14.78
16	do	do	do.[1]	15.19	13.17
17	do	do	do.[1]	15.33	13.13
8	do	Arkansas River Valley	do	17.39	15.45
11	do	do	do	18.84	16.74
2	Nancy Hall	Kansas River Valley	Early	21.06	19.45
13	do	do	Regular	21.50	19.42
18	do	do	do.[1]	18.96	16.59
7	do	Arkansas River Valley	do	21.49	19.73
9	do	do	do	19.69	17.93
3	Red Bermuda	Kansas River Valley	Early	13.84	12.85
12	do	do	Regular	14.50	13.57
14	do	do	do.[1]	14.87	13.42
6	Improved Big Stem Jersey	do	do	18.69	16.79
15	do	do	do[1]	17.83	15.60
10	Big Stem Jersey	Arkansas River Valley	do	18.94	17.14

[1] Cured sample.

SHED STORAGE VERSUS COLD STORAGE

Potato samples Nos. 11 and 12 were used in the storage experiments. The samples were identical in nature—Irish Cobbler, regular harvest, grade 2—and three 100-pound sacks were used for each experiment. Sample No. 11 was stored in a commercial cold storage plant in Manhattan, while sample No. 12 was placed in a shed of light frame construction, elevated on pilings to a height of about 5 feet above the ground. Of the three sacks of potatoes used in each experiment, one, designated "weight sample," was used only for determining the changes in weight (table 3) that occurred during the experiment; the second was used as the source of the analytical samples, and the third was held in reserve. On July 14, the weight of each weight sample was recorded and 10 pounds of potatoes were removed from the second sack of Nos. 11 and 12 for the preparation of the analytical samples. This procedure was repeated on the fourteenth of each

TABLE 3.—*Weight record on storage samples*

Month	Cold storage			Shed storage		
	Weight	Total loss		Weight	Total loss	
	Pounds	*Pounds*	*Percent*	*Pounds*	*Pounds*	*Percent*
July	90.0			89.5		
August	86.0	4.0	4.4	77.5	12.0	13.4
September	83.5	6.5	7.2	69.0	20.5	22.9
October	83.0	7.0	7.8	62.5	27.0	30.2
November	82.5	7.5	8.3	60.5	29.0	32.4
December	81.5	8.5	9.4	59.0	30.5	34.1
January	80.0	10.0	11.1	58.5	31.0	34.6

succeeding month to and including January 14, 1940. The analytical samples were handled in a manner subsequently described and analyzed with the other potato samples.

During the 6-month period the potatoes in shed storage were exposed to a wide range of conditions. While no attempt was made to keep a daily record of the temperature and humidity fluctuations, the monthly average for each of these factors as recorded by the Kansas State College weather observers is shown in table 4. The conditions to which the sample in cold storage was exposed were fairly constant. The company operating the storage plant stated that the temperature was maintained at 4.4° C. (±1°) and that previous determinations of relative humidity gave an average of about 85 percent for that part of the plant in which the samples were kept.

TABLE 4.—*Average temperature and humidity during the period of shed storage*

Month	Average mean temperature	Average relative humidity
1939	*°F.*	*Percent*
July	83.75	53.5
August	75.87	64.9
September	76.31	45.7
October	61.77	51.4
November	44.83	64.8
December	39.01	61.1
1940		
January	13.76	66.2

PREPARATION OF ANALYTICAL SAMPLES

Ten pounds of potatoes were taken from each of the incoming samples, scrubbed with a vegetable brush to remove all foreign matter, and dried by spreading on a wire screen before an electric fan. They were then cut into small pieces and passed four times through a large, dry meat grinder. No water was added at any stage of the preparation. The thick watery suspension was thoroughly mixed, 100 gm. weighed out on an analytical balance, and added to sufficient hot, acid-free, 95 percent ethyl alcohol to make the final alcoholic concentration—allowing for the water content of the potato—about 80 percent. Approximately 15 minutes was required for grinding and weighing the samples. The alcoholic suspension, contained in a fruit jar, was heated on a water bath for 30 minutes at a temperature slightly below the boiling point. The contents of the jar were thoroughly stirred at frequent intervals during the heating. The jars were then sealed, labeled, and stored until the next step in the preparation of the samples could be carried out.

Each sample was carefully filtered from the alcohol with suction on a Hirsch funnel. The residue was washed with about 150 cc. of 80-percent acid-free alcohol and dried as thoroughly as suction would permit. The funnel, containing filter paper and sample, was placed in an electric oven at 80° C. and kept there for 14 hours. The sample was carefully transferred to a tared weighing bottle, dried for an additional 3 hours at 80°, cooled in a desiccator, and weighed. This was taken as the weight of dry material that was obtained from 100 gm. of potatoes and that was insoluble in 80-percent alcohol. The dried material was ground to a fine powder and stored until needed for analysis. For the actual analysis, a small sample of each of these dry powders was placed in a tared weighing bottle, dried again at 80° for 2 to 3 hours, cooled in a desiccator, and reweighed.

METHOD OF ANALYSIS

A number of methods have been described as suitable for the determination of starch in vegetables. In most cases, satisfactory checks can be obtained as long as a given procedure is followed but rarely do the results obtained by any two methods check satisfactorily. The Association of Official Agricultural Chemists recommends two methods (*1, p. 342*). Both of these were used and the starch content is reported as determined by Direct Acid Hydrolysis, A. O. A. C. XXVII, 31, and by the Diastase Method with Subsequent Acid Hydrolysis, A. O. A. C. XXVII, 32.

The diastase used in the second method was Diastatic Spray Dried Malt No. 25, supplied by Eimer and Amend. This preparation contained 2 percent moisture. An infusion of the malt was prepared as needed. For each 80 cc. of malt extract required, 5 gm. of the malt powder was stirred for 20 minutes with water at room temperature. The extract was filtered and the clear brown infusion was used according to the directions given. Blank determinations on the enzyme preparations were run at frequent intervals and the experimental data corrected accordingly.

CALCULATION OF RESULTS

Table 5 shows a typical treatment of data obtained from the analysis of a sample of potato by the use of the direct acid hydrolysis method. In this case, the weight of the dry sample was 17.7798 gm.

TABLE 5.—*Sample of analytical data obtained in analysis of potato by the use of the direct acid hydrolysis method*

Weight of sample (grams)	$Na_2S_2O_3$	Cu_2O	Dextrose	Starch		
				Total	Dry weight	Fresh weight
	Cc.	*Mg.*	*Mg.*	*Mg.*	*Percent*	*Percent* [1]
1.5838	13.8	149.0	65.40	58.86	74.33	13.215
	13.9	150.1	65.94	59.35	74.95	13.325
	13.8	149.0	65.40	58.86	74.33	13.215
	13.9	150.1	65.94	59.35	74.95	13.325
1.6583	14.4	155.5	68.40	61.56	74.25	13.201
	14.5	156.6	68.90	62.01	74.79	13.297
	14.6	157.6	69.34	62.41	75.27	13.383
	14.5	156.6	68.90	62.01	74.79	13.297

[1] Average, 13.283.

In all cases, an accuracy of 0.2 cc. of standard sodium thiosulfate was required for the titrations to be accepted, and when calculating the averages only those values in which the percentage of starch (calculated to the original potato) checked within 0.2 percent were considered.

PRESENTATION AND DISCUSSION OF RESULTS

COMPARISON OF THE ANALYTICAL METHODS

There are several methods which have been used to determine starch on a quantitative basis and, while these methods may agree within themselves, they do not check with each other. Hence, it may be assumed that none of the known methods is an absolute measure of the starch content of any material. The two methods used in this work are accepted as probably the most accurate for this type of analysis. Since both methods were used with the same samples and under comparable conditions, an opportunity is presented to compare them.

The results obtained by the direct acid hydrolysis method were always higher than those obtained by the diastase method. In any given series of determinations the two sets of data showed the same trend—that is, an increase (or decrease) in starch content was reflected in the results obtained by both methods—but the values obtained by the two methods for any given sample did not agree. In the case of the potatoes, the starch content as determined by acid hydrolysis averaged 1.39 percent higher than the values obtained by the diastase method. The greatest difference observed was 1.94 percent, the least was 1.01 percent. With the cold-storage samples the range was from 1.12 to 1.49 percent with an average difference of 1.33 percent. The shed-storage samples showed a range of 1.21 to 1.59 percent, or an average of 1.43 percent. Considering all samples of potatoes the average difference was about 1.4 percent. The difference obtained when the sweetpotatoes were analyzed was noticeably greater, the average being 1.81 percent with a range of 0.93 to 2.37 percent.

Differences due to the method employed were expected since it has been generally accepted that the acid hydrolysis method may bring about the hydrolysis of substances other than starch while the enzyme diastase is thought to be relatively specific for starch. In this connection, however, a recent publication by Balch and Phillips (*2*) reports that malt diastase acts on certain nonstarch constituents of plant materials which are usually reported as starch.

It was believed, therefore, that further differences between the two methods would become apparent if they were used to estimate the starch values of pure starch samples. Hence, samples of pure starch were prepared from the sweetpotato and potato samples, analyzed by both methods, and the results compared with the analysis of the corresponding potatoes (table 6).

TABLE 6.—*Data for comparison of the methods of analysis*

Sample	Starch (dry-weight basis) obtained when analyzed by—		Difference	Deviation
	Acid hydrolysis	Diastase hydrolysis		
Sweetpotato No. 13:	*Percent*	*Percent*	*Percent*	*Percent*
Pure starch	86.50	81.05	5.45	6.7
Sweetpotato sample	78.20	70.64	7.56	10.7
Potato No. 11:				
Pure starch	79.57	75.09	4.48	6.0
Potato sample	76.42	69.14	7.28	10.5

These data confirm the prevalent opinion that there is a real difference inherent in the two methods of procedure. More specifically, they indicate that acid promotes more complete hydrolysis of starch than does diastase and that acid is more active than diastase in the hydrolysis of substances other than starch present in the tissues of potatoes and sweetpotatoes.

It is commonly assumed that the hydrolysis of starch, by either method, produces dextrose which is measured by the reduction of Fehling's solution. In the case, however, of incomplete hydrolysis, it is probable that maltose or some of the simpler polysaccharides would be present. Further, it is possible that under the conditions of the analysis -particularly if the reducing value of the hydrolyzed product were not determined immediately- a resynthesis of dextrose into some other disaccharide, or even simple polysaccharide, might occur. All of these substances might be reducing substances but their reducing index would certainly be lower than that of dextrose. Since the diastase method gave the lower values when used on the pure starch samples it appears that the carbohydrates present were not all in the form of glucose when the reduction of the Fehling's solution was carried out. Subsequent to the completion of these experiments, Etheredge (*5*) reported similar results with other pure starches. Not only did he find that these two methods yield different results but also that there was a lack of consistency with respect to which would give the larger and which the smaller starch values. In his experiments, the acid hydrolysis method indicated a 2.4 percent higher starch value for potato starch than did the diastase-acid method; this corresponds to a deviation of 6.0 percent in the present experiments.

After analysis of all the samples had been completed, rechecks were run in certain instances. In all cases it was much easier to duplicate former results when the acid hydrolysis method was used. No difficulty was encountered in obtaining checks within 0.1 percent, while with the diastase method the best that could be obtained was 0.2 percent.

Furthermore, when the diastase method is used it is necessary to run a blank determination on the malt extract and correct the titration values accordingly. In each instance the diastase solutions were prepared in exactly the same manner and a blank was made for every eight analyses. However, it is possible that slight variations in preparing or using the diastase solutions might produce a considerable difference in the degree of hydrolysis and it may be that a blank should accompany every determination.

After considering the reasons set forth above, it was concluded that the results obtained by the direct acid hydrolysis were the more satisfactory for making comparisons. Therefore, only these data will be considered in the remainder of the discussion.

INFLUENCE OF STORAGE ON STARCH CONTENT OF POTATOES

The potato storage results obtained in these experiments (table 7) are in general agreement with previous findings pertaining to effects

TABLE 7.—*Changes in weight and starch content of Kansas-grown potatoes during a 6-month period in cold storage and shed storage*

Month	Weight of solids	Starch			Weight loss of potatoes	Solids (fresh-weight basis)	Starch (fresh-weight basis)
		In solids	When analyzed by acid hydrolysis	When analyzed by diastase hydrolysis			
				COLD STORAGE			
	Grams	*Percent*	*Percent*	*Percent*	*Percent*	*Grams*	*Percent*
July	19.20	76.41	14.67	13.28		19.20	14.67
August	17.49	74.34	13.00	11.77	4.4	16.75	12.43
September	17.87	71.48	12.86	11.53	7.2	16.82	12.06
October	17.60	72.29	12.72	11.25	7.8	16.33	11.73
November	16.59	71.61	11.93	10.67	8.3	15.32	10.93
December	15.86	69.39	11.06	9.59	9.4	14.49	10.04
January	15.72	67.56	10.68	9.56	11.1	14.15	9.57
				SHED STORAGE			
July	19.20	76.41	14.67	13.28		19.20	14.67
August	19.36	75.16	14.55	13.34	13.4	17.07	12.60
September	20.89	74.75	15.63	14.17	22.9	17.00	12.05
October	20.24	74.69	15.11	13.56	30.2	15.53	10.54
November	19.54	74.83	14.66	13.24	32.4	14.62	9.73
December	20.44	74.93	15.35	13.86	34.1	15.25	10.12
January	21.20	73.60	15.79	14.20	31.6	15.74	10.32

of temperature and humidity changes on weight losses and on sugar-starch interconversion. The potatoes kept in cold storage over the 6-month period showed continuous and relatively gradual weight losses; the total loss was approximately 11 percent. The potatoes held in shed storage, which were subjected to radical changes in temperature and relative humidity, suffered continuous losses in weight but the rate of loss fell off markedly toward the end of the storage; the total loss was approximately 35 percent.

It may be shown graphically that changes in starch, alcohol-insoluble solids, and starch in solids from cold-storage potatoes follow approximately the same trend although there is some reason to believe that these values have responded to the variable humidity of storage. A similar treatment of shed-storage data shows a definite correlation between the changes in starch and solids. This relationship is not due, as might be expected, to a similar variation in the starch content of the solids, since there is little change in the latter between the second and fifth months. When starch values are corrected to the original weights of the potatoes, it becomes evident that there is little to choose between cold storage and shed storage, judging from the over-all losses of starch as determined by analysis; in either case, there has been a drop of from 4 to 5 percent.

One interesting relationship, more or less incidental to the practical aspects of the problem, is that observed between the starch or solids content and the water content of shed-storage potatoes as reflected by the temperature, or even better by the temperature-relative humidity ratio, at the time of sampling. Figure 1 shows graphically how changes in starch correspond to sharp changes in this ratio.

It is believed that this relationship may be explained on the basis of a dependence of the tissue-water concentration on environmental factors that affect transpiration. Other principal factors which may produce an increase in tissue-water concentration are the sugar-starch conversion and respiration. However, the latter changes are opposing reactions in the sense that each is dependent upon the sugar concentration. It is conceivable that there may be conditions under which the relative rates of change in the sugar-starch conversion and respiration may be such that there is relatively little sugar available for respiration. In such a case, an appreciable part of the water replacing that lost through transpiration would come through the conversion of sugar to starch. With subsequent lowering of the temperature-relative humidity ratio, the tissue-water concentration could increase through respiration and, as it does, permit a proportionate shift from starch to sugar. In such a manner, starch could increase or decrease with change in the temperature-relative humidity ratio.

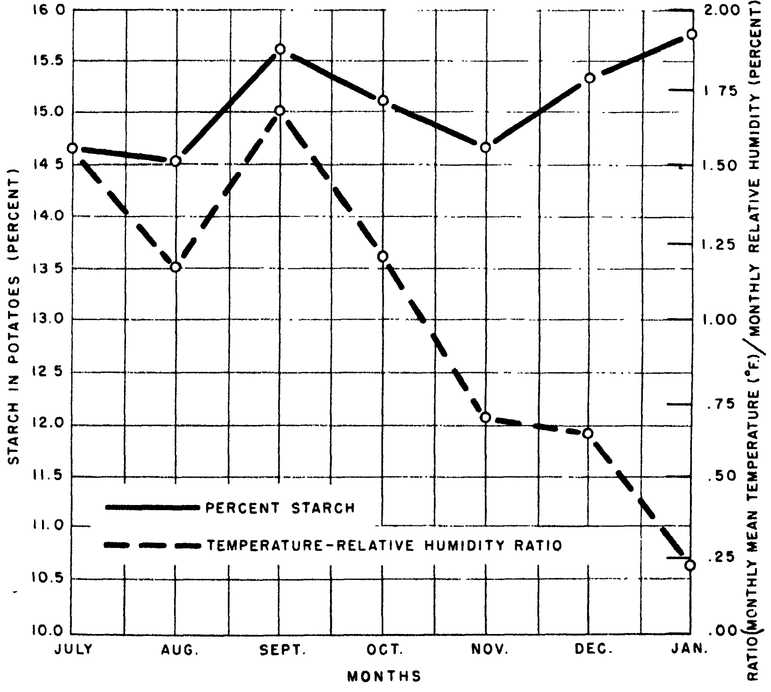

Figure 1.—Changes in the starch content of potatoes and in the temperature-relative humidity ratio during shed storage.

The above explanation is supported in some measure by evidence recently presented by Barker (*3*) that, in potatoes, sucrose either is or determines the respirable substrate. Barker found that, within the temperature limits of 1° to 10° C. and excepting high sucrose concentrations, curves relating the rate of respiration with the concentration of extractable sucrose closely resemble those which characterize the rate of reaction-substrate concentration of enzymic reactions in vitro. Although it must be recognized that higher temperatures might alter the form of respiration-substrate curves, presumably because of lower sugar concentrations, it should be manifest that the water concentration plays an important part in the balance mechanism existing in plant tissue whatever the prevailing conditions may be. Accordingly, those environmental factors which may influence the tissue-water concentration may indirectly affect the sugar-starch balance in the tissue.

INFLUENCE OF VARIETY, HARVEST PERIOD, AND SOIL TYPE ON STARCH CONTENT OF POTATOES AND SWEETPOTATOES

The starch content of the various varieties is well illustrated by the data obtained on samples 6, 7, and 8 (shown in the middle column of table 8). These were samples grown under the same conditions, on the same soil, and harvested at the regular period. Inspection of table 8 shows that the Irish Cobbler had the greatest starch content, with Warba next and Bliss Triumph the least. This same trend may be observed throughout the other samples used.

Table 8 also shows the effect of the harvest period on starch content.

TABLE 8.—*Effect of variety and harvest period on starch content of potatoes*

Variety	Starch (fresh-weight basis) when harvest was—		
	Early	Regular	Late
	Percent	*Percent*	*Percent*
Irish Cobbler	14.67	15.41	13.30
Warba	12.59	13.56	11.82
Bliss Triumph	10.99	11.45	9.81

The starch content is highest at regular harvest, slightly lower at early harvest, and still lower at late harvest periods. Apparently, so far as starch is concerned, the harvest could be started a week earlier, but some loss would result if the harvest were delayed much past the regular period. If the crop weight loss is considered, however, early harvesting becomes as impracticable as late harvesting. Regular harvest has the double advantage of maximum starch content and maximum crop weight.

While it is thought that the type of soil and the previous crop are factors which would have some effect on the starch content of potatoes, the number of samples examined was not sufficient to permit definite conclusions to be drawn. It was observed that at regular harvest and with potatoes as the previous crop, Irish Cobblers contained 15.19 percent starch when grown on upland soil, 15.41 percent on fine sandy loam, and 13.28 percent on loamy sand. Moreover, regular-harvest Irish Cobblers when grown on fine sandy loam contained 14.31 percent starch when the previous crop was oats (followed by sweetclover, which was a failure), 14.64 percent when the previous crop was alfalfa, and 15.41 percent when the previous crop was potatoes. These trends would have to be supported by additional evidence before it would be safe to draw any generalizations.

Varietal differences were very noticeable in the samples of sweetpotatoes analyzed. In all cases (table 9) the Nancy Hall contained the highest percentage of starch and Red Bermuda the least, with Little Stem Jersey and Big Stem Jersey intermediate. In this connection, it is worthy of note that the Mississippi Agricultural Experiment Station in 1936 (7, *p. 1342*) found the Nancy Hall to contain 22.0 per cent of starches as compared with the 21.5 percent shown in table 9.

TABLE 9.—*Variety comparison with sweetpotatoes from different producing areas in Kansas*

Variety	Starch (fresh-weight basis) in sweetpotatoes from—	
	Kansas River Valley	Arkansas River Valley
	Percent	*Percent*
Nancy Hall	21.59	21.49
	------	19.69
Improved Big Stem Jersey	18.69	------
Big Stem Jersey	------	18.94
Little Stem Jersey	16.88	18.84
	16.57	17.39
Red Bermuda	14.50	------

Some variation was observed between the potatoes grown in the Kansas and Arkansas River Valleys, but this also could be attributed to other factors.

A slight increase in starch content was observed in the regular-harvest samples over the early-harvest samples. Also, as would be expected, the curing process brought about a decrease in starch content (table 10).

TABLE 10.—*Effect of harvest period and curing* [1]

Variety	Starch in sweetpotatoes		
	Early harvest	Regular harvest	Cured
	Percent	*Percent*	*Percent*
Nancy Hall	21.06	21.59	18.96
Improved Big Stem Jersey	------	18.69	17.83
Little Stem Jersey	15.21	16.57	15.33

[1] All samples from Kansas River Valley.

Among the sweetpotato varieties, the Nancy Hall contained the most starch and Red Bermuda the least, with Big Stem Jersey and Little Stem Jersey intermediate.

No important difference was observed between the sweetpotatoes grown in the Kansas River Valley and those grown in the Arkansas River Valley.

The regular-harvest sweetpotatoes had a slightly higher starch content than the early-harvest sweetpotatoes.

Curing of sweetpotatoes caused a decrease in starch content.

SUMMARY AND CONCLUSIONS

Thirty-six samples—18 of potatoes and 18 of sweetpotatoes—were analyzed by the direct acid hydrolysis method and the diastase hydrolysis method of the Association of Official Agricultural Chemists.

Studies on the relative effectiveness of cold storage and shed storage for potatoes showed that the decrease in starch was about the same in both, namely between 4 and 5 percent. Moisture loss of the shed-storage sample amounted to about 35 percent of its original weight as compared with a loss of about 11 percent for the cold-atorage sample.

Varietal differences in potatoes indicated the order of starch content to be Irish Cobbler, Warba, and Bliss Triumph.

The starch content of the late-harvest potatoes was decidedly lower than that of either the regular- or the early-harvest potatoes. No pronounced difference was observed in these last two groups. When the crop weight loss of early-harvest potatoes is considered, it is obvious that a certain stage of maturity must be reached before harvesting begins.

As to soil types, the sandy loam seemed to be slightly better than loamy sand, but the results are not to be regarded as conclusive.

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EFFECT OF STORAGE CONDITIONS ON THE VIABILITY OF TOBACCO SEED[1]

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INTRODUCTION AND REVIEW OF LITERATURE

Seed of cigar-wrapper tobacco stored in ordinary rooms in Florida for more than 2 or 3 years seldom remains sufficiently viable to plant. Farmers who sow seed older than this without testing the germination are often disappointed in the resulting stand and vigor of the seedlings. Likewise, in Puerto Rico, "the Tobacco Institute does not recommend the use of tobacco seed after the second year."[2]

On the other hand, tobacco seed has been reported to retain its viability for much longer periods in colder climates. Johnson, Murwin, and Ogden (*4*)[3] reported that in Wisconsin, "seed that was originally of good germinating capacity may be quite satisfactory for commercial purposes when 10 years old." It is reported (*2*) that in Connecticut, "high testing seed in general shows very little diminution in germinative capacity up to 5 years; after that the percentage usually falls gradually, although occasionally there is excellent germination at 10 years."

Shamel and Cobey (*7*) stated that "thoroughly dry seed may be shelled and stored in glass vials or bottles with perfect safety, and can be kept almost indefinitely in this way; the fully matured and dry tobacco seed will retain its viability when kept dry for 10 years, or, as has been observed in several cases, a much longer time."

Chirkovskiĭ (*1*) reported that optimum storage conditions for tobacco seed are low temperature, but above freezing, and low relative humidity.

Poptzoff (*6*) found that the higher the storage temperature used for tobacco seed, the lower must be the relative humidity. He further claims that there is no such thing as a lower limit of relative humidity, for the lower it is, the greater the certainty of conserving the quality of the seed.

EXPERIMENTAL WORK

An experiment to determine some of the factors affecting the longevity of tobacco seed in storage was started at Quincy, Fla. in August 1931 with freshly harvested seed of a cigar-wrapper variety, No. 301. The original moisture content of the seed was estimated by drying to constant weight at 102° C.

Three series of tests were conducted, as follows:

1. Seed was stored in small vials enclosed in 4-ounce screw-cap glass jars over various chemicals, which kept the relative humidity of the air fairly constant at values determined from chemical reference works. The chemicals used were anhydrous calcium chloride ($CaCl_2$), Rochelle salt ($KNaC_4H_4O_6 \cdot 4H_2O$), ferrous sulfate ($FeSO_4 \cdot 7H_2O$), a

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[1] Received for publication February 2, 1943.

[2] Letter from Dr. H. H. Foster, dated June 1, 1942.

[3] Italic numbers in parentheses refer to Literature Cited, p. 410.

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saturated solution of ammonium nitrate (NH_4NO_3) with an excess of the salt, and water.

2. Seed was stored in rubber-stoppered vials after the moisture content, originally about 9 percent, had been adjusted to various percentages by placing samples in a moist chamber or a desiccator for various lengths of time. No attempt was made to restore the moisture content to its original value each year when the vials were opened for sampling.

3. Seed with the original moisture content was stored in rubber-stoppered vials, paper envelopes, and cloth bags, and kept in various locations, as follows: Refrigerator, electric, fairly constant at 5° C.; basement room, unheated; laboratory, intermittently heated in winter; and an attic, cold in winter and extremely hot in summer.

No attempt was made to control the amount of light received by the seed during storage. All containers were exposed to diffused daylight at least part of the time.

In October 1932 and at intervals of about a year thereafter, each lot of seed in storage was poured out, mixed, and sampled for the germination test. Any desiccants which appeared to have deteriorated were renewed, and the seeds were returned to storage.

Tests were made on triplicate samples of 100 seeds each, in Petri dishes on two sheets of wet filter paper. The light requirement for the germination of these seeds at constant temperature (*5*) was satisfied by opening the incubator nearly every day. The testing procedure provided favorable conditions for germination, as shown by the fact that every year some tests averaged 80 percent or more. At convenient intervals of a few days, seedlings were counted and removed from the dishes, until further incubation of a few days gave little or no further germination. The averages of triplicate germination tests for each of the 11 years of the experiment to date are given in table 1.

Seeds stored in the refrigerator over the three salts, calcium chloride, Rochelle salt, and ferrous sulfate, retained their viability for 11 years. Those stored over a saturated solution of ammonium nitrate deteriorated considerably, and those stored over water were all dead at the end of 1 year.

Seeds stored in either the basement, laboratory, or attic over calcium chloride retained their viability for 11 years. Those stored over the other salts or the salt solution were all dead after from 2 to 5 years, and those stored over water were all dead at the end of 1 year

Seeds stored in stoppered vials with an original moisture content of 5.3 percent or less showed a small percentage of germination after 8 years; they would probably have remained viable much longer if the original moisture content had been restored at suitable intervals. With each increment in original moisture content the survival period decreased, until at 10.7 percent moisture, nearly all the seeds were dead after 1 year.

Seeds stored in stoppered vials, paper envelopes, or cloth bags kept well in the refrigerator, but in the other three locations all or nearly all were dead after 3 years.

Samples from each of the nine lots of seed which germinated 79 percent or more after 10 years in storage were sowed in an ordinary outdoor plant bed in 1942. The stand and growth appeared just as good as were obtained with fresh seed of the same variety.

TABLE 1.—*Germination of tobacco seed after storage under various conditions and for various periods*

How stored	Where stored	Approximate relative humidity inside container	Original moisture content of seed	Germination after number of years indicated										
				1	2	3	4	5	6	7	8	9	10	11
Over chemicals:		*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
$CaCl_2$	Refrigerator	1	9	85	50	23	58	76	73	48	78	84	82	88
$KNa(C_4H_4O_6).4H_2O$	do	30	9	86	45	19	60	87	74	67	85	80	85	89
$FeSO_4.7H_2O$	do	[1] 50	9	79	34	23	58	80	70	55	86	86	88	82
NH_4NO_3 [2]	do	70	9	80	70	63	70	73	67	51	58	49	51	42
H_2O	do	100	9	0										
$CaCl_2$	Basement	1	9	65	54	53	69	86	84	81	87	87	91	86
$KNa(C_4H_4O_6).4H_2O$	do	40	9	51	0	0	0							
$FeSO_4.7H_2O$	do	60	9	65	6	0	0							
NH_4NO_3 [2]	do	60	9	81	60	47								
H_2O	do	100	9	0										
$CaCl_2$	Laboratory	1	9	78	52	46	76	57	83	72	75	87	83	79
$KNa(C_4H_4O_6).4H_2O$	do	40	9	77	45	30	1	0						
$FeSO_4.7H_2O$	do	60	9	82	49	40	11	0						
NH_4NO_3 [2]	do	60	9	83	55	60	3	0						
H_2O	do	100	9	0										
$CaCl_2$	Attic	1	9	81	56	60	80	82	79	77	81	74	79	83
$KNa(C_4H_4O_6).4H_2O$	do	40	9	76	54	43	0	0						
$FeSO_4.7H_2O$	do	60	9	67	16	2	0							
NH_4NO_3 [2]	do	60	9	81	31	0	0							
H_2O	do	100	9	0										
In stoppered vials	Laboratory		2.8	89	63	84	77	62	27	14	2	0		
	do		4.3	80	71	79	68	66	36	7	3	0		
	do		5.3	92	78	80	77	69	46	12	11	0	0	
	do		6.8	87	80	68	41	18	3	0				
	do		8.0	79	64	57	16	0						
	do		9.0	70	25	0	0							
	do		10.7	2	0	0	0							
In various containers:														
Stoppered vial	Refrigerator		9	76	49	24	60	89	75	65	81	83	86	83
Paper envelop	do		9	77		14	35	85	68	69	81	82	85	84
Cloth bag	do		9	61		20	70	78	77	70	77	83	81	73
Stoppered vial	Basement		9	63	8	0	0							
Stoppered vial	Laboratory		9	60	16	0	0							
Paper envelop	do		9	63	28	1	0							
Cloth bag	do		9	72	29	2	0							
Stoppered vial	Attic		9	49	1	0	0							

[1] Estimated from values found for $CoSO_4.7H_2O$.
[2] Saturated solution.

It is planned to continue the experiment at least as long as any of the samples keep satisfactory viability.

DISCUSSION

Practical methods for storing tobacco seed for 11 years, perhaps much longer, are suggested by the results of this experiment.

Ordinary desiccators containing calcium chloride, or probably any other good desiccant, may be used. A desiccator can be improvised from any tight container of glass or metal, using anhydrous calcium chloride and a false bottom to support the seed. Drying the seed in a desiccator and then storing it in a tight container would probably be satisfactory.

Storage in an electric refrigerator at a temperature a few degrees above freezing, with the seed enclosed in ordinary containers, gave as good results as dry storage at any temperature tested. Storage in an ice refrigerator would probably not be as satisfactory, because of the higher humidity and usually higher temperature.

Farmers who produce a supply of tobacco seed sufficient for several years may preserve it for many years by suitable storage. Such a supply of seed may be valued more highly after one or more satisfactory crops have been produced from it. The importance of being able to preserve experimental lots of tobacco seed for long periods need not be discussed here.

Several samples, especially those stored in the refrigerator, showed a decline in germination test during the first 3 years in storage, and afterwards returned to a high test. Similar results have been reported by Goodspeed (*3*) and by Johnson, Murwin, and Ogden (*4*). No explanation can be offered, but it seems improbable that this kind of result was due to the conditions of the germination tests, because every year some of the tests averaged 80 percent or more. Another storage experiment has been started, in which this phenomenon will be given special attention.

SUMMARY

Cigar-wrapper tobacco seed of the 1931 crop was placed in storage at Quincy, Fla., immediately after harvest (1) in closed containers over various chemicals, (2) in rubber-stoppered vials with the moisture content of the seed adjusted and determined, and (3) in various containers. Test lots were kept in different locations, as follows: Refrigerator at about 5° C., basement, laboratory, and attic.

After 11 years, seed stored over calcium chloride in each location, and seed stored in various ways in the refrigerator, germinated well in the laboratory. Samples which tested 79 percent or more after 10 years also germinated well in an ordinary outdoor plant bed.

Seed stored in rubber-stoppered vials with an original moisture content of 5.3 percent or less showed a small percentage of germination after 8 years.

Samples stored in ordinary containers in the laboratory were all or nearly all dead after 3 years.

Practical applications of dry or cold storage to commercial and experimental lots of tobacco seed are suggested.

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THE EXCRETION OF ARSENIC BY THE MALPIGHIAN TUBES OF GALLERIA MELLONELLA, TENEBRIO MOLITOR, AND RHODOPHORA FLORIDA[1]

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INTRODUCTION

The work herein presented formed part of a program of research dealing with the physiological factors in insect control with insecticidal materials. The ultimate goal of this program is the determination and estimation of basic physiological factors and the improvement of control practice according to the factors involved. As a starting point the elimination of arsenious ion by the organs of excretion was investigated. The rates of absorption of a solution of sodium arsenite of lethal concentration in normal saline were determined and the effects of the arsenious ion upon the activity of certain enzyme systems were assayed.

The specific purpose of the investigation was to estimate the part played by the excretory system of insects of different degrees of susceptibility in the elimination of arsenic.

PREVIOUS WORK

Several attempts have been made to determine how arsenic acts on insects and why some insects are resistant to it. Fink (*3*)[2] measured the respiratory rates and the respiratory quotients of potato beetles and wireworms which had been fed various arsenicals, and found that in general the oxygen intake was reduced and the respiratory quotient was increased. Following this study Fink (*4*) determined the effects of arsenic upon the glutathione content of a series of insects. He found the content reduced with arsenic poisoning, but observed that in *Malacosoma americana*, his most resistant species, the initial glutathione content was the lowest. In a third series of investigations, Fink (*5*) studied the activities of arsenic upon amylase, lactase, maltase, invertase, lipase, and protease from potato beetles. No significant effects were observed in the presence of paris green and lead arsenate. Parfentjev and Devrient (*9*) observed no significant changes in the respiratory quotient of cockroaches poisoned with arsenic, and the respiration of tissues (muscle, midgut, and nerve tissue) dipped in a solution containing a lethal concentration of arsenic showed no significant reduction. Voskresenskaya (*12*) described the effects of sodium arsenite upon the rate of passage of food through the alimentary canal, its periodic concentration within the tissues of the insect, and the effects of arsenic upon the anterior sphincter of the midgut. She concluded that resistance depends largely upon the

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ability of the insect to regurgitate the dose that has been taken in with the food and to maintain a constant relation between the rate of absorption and the rate of elimination of the material from the blood.

MATERIALS AND METHODS

Three species of insects were used in this study, namely, last instar larvae of *Galleria mellonella* L.; late instar larvae of *Tenebrio molitor* L., both of these from laboratory cultures; and last instar larvae of *Rhodophora florida* Gn., a noctuid collected from its host plant, the evening primrose.

The median lethal dose (M. L. D.) of the arsenious ion was determined for *Galleria mellonella* and *Tenebrio molitor* by buccal injection of measured doses. The solution used was prepared from reagent grade (Baker's) arsenious oxide and sodium hydroxide. The pH of the final product was adjusted to neutrality. The sigmoid mortality curve derived from these measurements was rectified by the method of probits described by Bliss (*1*). Unfavorable weather conditions prevented the collection of sufficient numbers of *Rhodophora florida* to make possible the construction of mortality curves.

The rate-of-absorption measurements were made by the method described by Patton and Craig (*10*). In order to eliminate the factor of individual variation, two absorption gages were used simultaneously with each insect, one containing normal saline and a loop of Malpighian tube and the other containing a symmetrical loop from the same larva and a saline made 0.01 N with sodium arsenite.[3] The normal saline was prepared in a stock solution by dissolving 1.95 gm. of NaCl, 1.52 gm. of KCl, and 1.00 gm. of $CaCl_2$ in distilled water and making up to a volume of 100 ml. at 20° C. The pH was adjusted to neutrality by adding sodium bicarbonate or hydrochloric acid. This stock solution was adjusted to an osmotic pressure nearly corresponding to that of the normal blood of the insect under investigation. The osmotic pressure values were determined by the capillary method of Halket (*6*) for the lepidopterous larvae and the values were taken from previous work (Patton and Craig (*10*)) for the mealworm. The osmotic pressure of the saline was adjusted by dilution from a curve prepared by determining the freezing-point depression of several known dilutions of the stock saline.

Data on selective absorption were obtained by measuring the electrical conductivity of the solution in the bulb of the gage at regular intervals over a period of 24 hours. Measurements were made by means of a conductivity bridge.

The recovery of arsenic in the hind-gut was determined by inserting a short section of No. 20 white cotton thread incased in a finely drawn glass tube into the hind-gut through the anal opening of the insect during an absorption measurement. Analysis was subsequently made on the thread by a micro Gutzeit method essentially the same as that of Howe (*8*).

Estimation of the presence of certain of the respiratory enzyme systems (monophenol, polyphenol, and indophenol oxidase, peroxidase, and glutathione), and of the autolytic activity of a protease was made by using well-known colorimetric tests such as are enumerated by Hawk and Bergeim (*7*). The protease activity of the tissues comprising the tubes was estimated from the free amino acid content of an incubated brei by the Danielson modification of the method of Folin (Hawk and Bergeim (*7*)).

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[3] This constitutes a calculated lethal concentration based upon figures published by Yeager and Tauber (13) on the blood volume of *Periplaneta fuliginosa*, and by Fay (*2*) on lethal arsenic content of the blood of *P. americana*.

RESULTS

As a result of a series of measurements of the rates of absorption, it was demonstrated that in all species upon which observations were made, arsenic was readily taken up by the Malpighian tubes. In *Galleria mellonella*, the more susceptible species (M. L. D.$=9\gamma$ (0.009 mg.) As^{+++} per gram live weight), it was possible to show a slight decrease in rate of absorption in the presence of arsenic. When these data were evaluated statistically by the method of individual comparison according to Snedecor (*11*), the *Z* chart test showed that the significance of the reduction in rate was barely within the 5 percent level. (Mean difference$=0.042$, standard deviation$=0.149$, and the number of pairs$=48$.) Significance could not be established between differences found in the rates of absorption of normal and arsenic-bearing saline with either *Tenebrio molitor* or *Rhodophora florida*. (M. L. D. for *T. molitor*$=12\gamma$ (0.012 mg.) As^{+++} per gram; for *R. florida* the M. L. D. was undetermined but is known to have been less than 15γ (0.015 mg.) As^{+++} per gram.)

The qualitative survey of the effects of arsenic upon respiratory enzyme activity showed no appreciable change in the activity of the enzymes demonstrated to be present in the normal Malpighian tissues. No monophenol oxidase, polyphenol oxidase, or glutathione could be demonstrated in any case. The presence of arsenic appeared to have no effect upon the activity of indophenol oxidase, which gave a weak test with Nadi[4] reagent in both treated and untreated samples, or upon the activity of the peroxidase present.

A comparison of the relative concentrations of free amino acid in salines containing nearly equivalent amounts of Malpighian tissue was made after an incubation period of 3 hours. The tubes of *Galleria mellonella* and *Tenebrio molitor* showed nearly equal concentrations of free amino acids in both samples. Apparently protein autolysis is not significantly inhibited by the presence of As^{+++}.

That the absorption of arsenic by the tubes is not selective in *Galleria mellonella* and *Tenebrio molitor* was demonstrated by the fact that the electrical conductivity of the saline bathing the tubes was unchanged over a period of 24 hours. During this time an average total volume of 4.9λ (cubic millimeters) of solution was absorbed.

Analysis of the threads which had been inserted into the hind-gut of the larvae of *Galleria mellonella* showed an average recovery of 38 percent of the (calculated) arsenic absorbed by the tubes. Difficulty in collecting the samples made quantitative recovery impossible. Anatomical differences precluded comparable measurements with *Tenebrio molitor*.

DISCUSSION

The results of the foregoing experiments demonstrate that the Malpighian tubes of all three species of insects used in this study were able to filter the body fluid of the insect and remove soluble salts by a process comparable to the action of vertebrate glomeruli. They also demonstrate that the presence of arsenious ion in a concentration which approximates the lethal concentration has no significant effect upon the ability of the tubes to function in the process of filtration. A lethal dose of arsenic administered buccally to a series of mealworms which were subsequently dissected and the rates of absorption of normal saline determined caused no significant change of rate.

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[4] Equal quantities of 1 percent alpha naphthol (in 95 percent alcohol) and 1 percent paraphenylenediamine (in water).

From these data it is apparent that the Malpighian tubes play a definite role in the removal of arsenic from the blood stream of insects poisoned by the ingestion of arsenic-bearing insecticides. There is no significant relation between the susceptibility of the insect and the effects of arsenic upon the tubes.

SUMMARY

The rates of absorption of arsenious ion by the Malpighian tubes of three species of insects—*Galleria mellonella*, *Tenebrio molitor*, and *Rhodophora florida*—were determined and compared. *G. mellonella*, the species most susceptible to arsenic poisoning, showed a reduction in the rate of absorption in the presence of arsenic but the reduction was of indeterminate significance. The other two species showed no significant difference. None of the enzyme systems estimated were affected.

From the measurements made, it is apparent that the Malpighian system of insects plays a definite role in the elimination of arsenic from the blood; however, the data give no indication that the function of these organs is the important factor in the relative susceptibility of insects to arsenic poisoning.

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SEASONAL DEVELOPMENT IN THE NURSERY OF DAMPING-OFF OF RED PINE SEEDLINGS CAUSED BY PYTHIUM AND RHIZOCTONIA[1]

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INTRODUCTION

A detailed study of the weather and certain soil conditions in relation to damping-off of red pine seedlings in Wisconsin forest nurseries has been undertaken. The present paper is the third of a series on the damping-off of red pine seedlings by *Pythium* and *Rhizoctonia*. In the first paper (*6*) [3] the causal agents were shown to be *Pythium irregulare* Buisman and *Rhizoctonia solani* Kühn and their distribution, symptoms caused, and life histories were presented; in the second paper (*7*) the influence of controlled temperature, moisture, and soil reaction was described. Several interacting factors have operated at various times and in different places to favor or to inhibit damping-off in the nursery. The study here reported attempts to clarify the outcome when all these factors act freely together in nature.

The reaction of the soil has appeared to be a very important influencing factor. Hartley (*3*) has observed that in the nursery severe damping-off was highly correlated with neutral or only slightly acid soils. Damping-off was not serious on acid soils where the pH was less than 6. Roth and Riker (*7*) have reported that damping-off is somewhat diminished in the greenhouse in soils of about pH 5 but that deviation from this reaction in either direction may increase the severity of attack where both *Pythium* and *Rhizoctonia* are the causal fungi.

High soil moisture and air humidity, it is commonly assumed, favor damping-off. This view was partly upheld by Hansen et al. (*2*), who studied damping-off in seedbeds receiving different amounts of water. They found that, in general, but not always, heavy watering increased the amount of damping-off. C. Roth (*5*) considered wet soil and high air humidity favorable to damping-off by *Rhizoctonia* during periods of hot weather. However, Hartley (*3*) observed that in a Nebraska nursery damping-off decreased after heavy rains and did not increase again until soil moisture had gone down. Roth and Riker (*7*) found that various damping-off fungi responded differently in the greenhouse to soil moisture, *Pythium* thriving in wet soil and *Rhizoctonia* causing greater losses in somewhat drier soils and at saturated air humidity.

That high temperatures favor damping-off was pointed out by Jones (*4*), Hartley (*3*), and C. Roth (*5*). Hartley and C. Roth interpreted their data by placing the values for total damping-off at differ-

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[3] Italic numbers in parentheses refer to Literature Cited, p. 481.

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ent intervals after emergence in juxtaposition to meteorological records obtained during the same period. Hartley counted damped-off seedlings twice daily in naturally infested beds, but in presenting his data he made no statement regarding the kinds of damping-off fungi present. C. Roth's (*5*) studies were made on beds inoculated with *Pythium*, *Fusarium*, and *Corticium* (*Rhizoctonia*), but his discussion of results mentioned only *Corticium* damping-off. In greenhouse studies in soils inoculated with *Pythium* and *Rhizoctonia*, Roth and Riker (*7*) found that warm temperatures favored damping-off by both fungi. When the soil was wet, *Rhizoctonia* was strongly inhibited by cool temperatures while *Pythium* continued to cause severe losses.

Clarifying the effect of environmental factors on disease development involves several considerations: (1) Temperature and moisture often vary simultaneously, to complicate results. Field plots can seldom be controlled to the point where an effect may be attributed to a single variable. (2) The reaction of the soil influences the activity of the causal fungi, *Rhizoctonia* predominating in soil more acid than approximately pH 5.8 and *Pythium* in that less acid. (3) The identity of the active damping-off fungi must be known since the various causal agents differ (*5*, *6*) in their response to environment. Information that has been obtained where one organism is acting cannot be applied where the loss is caused by a different organism. (4) Age of the host plant strongly affects its susceptibility (*3*), prohibiting direct comparison of the results of one count with those of a subsequent count of the same stand. (5) Age differences influence the time required for symptom development (*3*). Thus, in older seedlings when counts are made at frequent intervals, the effect of an attack one day may not appear until a day or more later. (6) Various host plants differ in susceptibility (*3*, *9*) while various strains of the fungi differ in pathogenicity (*3*, *6*). Results obtained with a particular host and parasite must, therefore, be applied with reservation to a different disease situation.

With these various considerations in mind the writers have examined the seasonal expression of damping-off in a nursery in central Wisconsin and have interpreted the relative importance of the different interactive influences involved. It appeared that a clarification of interacting environmental factors might help explain the variation in the occurrence of damping-off and in results secured with different control measures from year to year in the same nursery and from nursery to nursery during the same year.

1938 EXPERIMENTS

MATERIALS AND METHODS

The experimental area was a uniform nursery block, provided with overhead irrigation and located some distance from roads or windbreaks. It had been reclaimed from an abandoned field in 1932 and seeded to white pine in 1933. Damping-off in this first planting was so severe that the area was subsequently used only for transplanting. The soil was Plainfield sand of uniform structure and low but uniform fertility. The reaction was pH 5.5.

Sixteen standard 4- by 12-foot seedbeds were placed in a rectangle with 4 beds to a side. Each bed accommodated five 2- by 4-foot experimental plots having 6-inch separations between plots. This arrangement allowed 4 replications of 20 treatments (seeding dates). The replications, each consisting of 1 row of 4 beds, were placed side by side, and the 20 plots within each replication were completely randomized. Each bed was covered with a bird- and rodent-proof frame providing half shade.

The soil of each 2- by 4-foot plot was uniformly inoculated by thoroughly mixing into the upper 3 inches one-third quart of a corn meal and sand culture of *Rhizoctonia* (F–5) and a similar amount of *Pythium* (F–111–A), described earlier (*6*). The plots were seeded 5 days after inoculation.

Seed employed in 1938 and 1939 was from a uniform, locally produced lot of red pine (WR–17). It germinated 91.3 percent in a bed of sterile sand adjacent to the experimental plots.

Complications arising from age differences of the host were reduced by providing throughout the summer a population of seedlings of uniform age. This supply was obtained by seeding a new set of four plots (one in each replication) every 5 days.

Approximately 1,450 seeds were planted in each plot consisting of twelve 4-foot drills. The 2 outer drills and 3 inches of the ends of the 10 inner drills were omitted from counts to avoid marginal effect. Counts were taken on 20 feet chosen by marking 2 linear feet from alternate ends of each of the 10 inner drills of the plots and included about 550 viable seeds.

Meteorological records were made at the plots throughout the summer. Instruments used were a standard drum-recording hygrothermograph and a drum-recording soil thermograph with the bulb buried one-quarter inch in one of the experimental plots. Rainfall and water thrown by the overhead sprinklers were measured with a standard rain gage. The moisture content of the uppermost inch of soil was measured daily at 8 a. m. in three composite samples taken at random from the experimental area. The soil reaction was measured periodically on composite samples with the quinhydrone electrode. Occasional samples were also tested with a glass electrode to check the efficiency of the other instrument.

The experiment was so designed that seedlings in the four plots of the first seeding were counted 5 days after emergence and, thereafter, at 5-day intervals until six counts had been made. The seedlings had then passed their age of practical susceptibility. The second and later seedlings were treated in like manner throughout the summer. Thus a succession of damping-off and survival counts was provided of seedlings comparable in age but variously affected by environment. Preemergence damping-off has been omitted from the records of results. A series of estimates indicated that in the field preemergence damping-off had approximately the same ratio to postemergence damping-off as under similar but controlled conditions in the greenhouse (*7*).

A representative sample of damped-off seedlings was collected on each counting date from the plots of each age group. A section of the hypocotyl from each seedling was plated and the causal fungus determined. The numbers of seedlings plating *Pythium* and *Rhizoctonia*, respectively, were recorded. The causal fungi could be consistently isolated only from seedlings damped off 1 to 3 days preceding

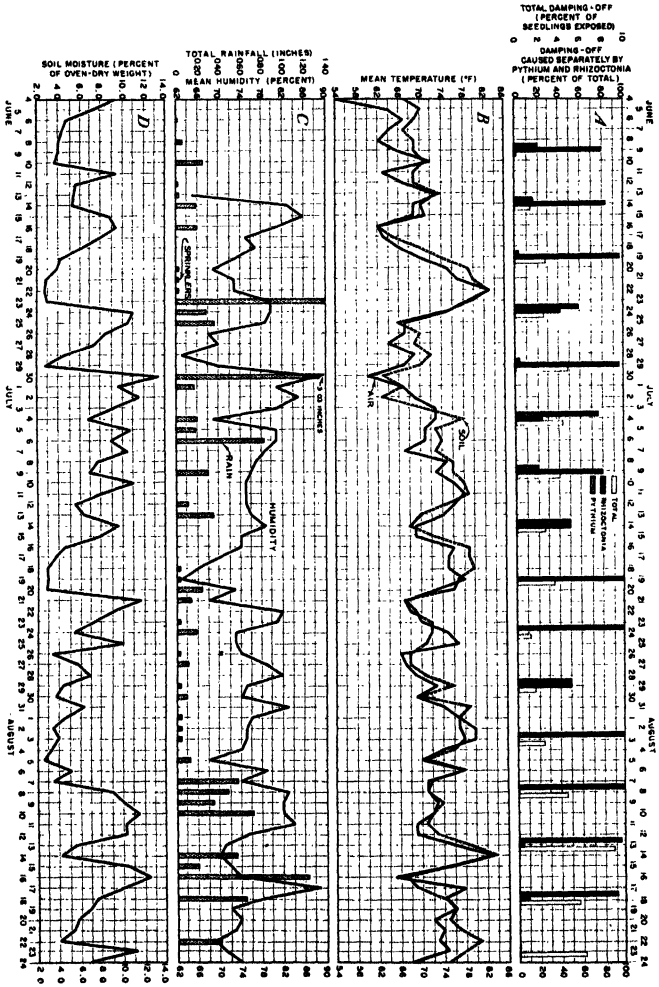

FIGURE 1.—Damping-off of red pine caused, respectively, by *Pythium* and *Rhizoctonia* during the summer of 1938 in inoculated field plots on Plainfield sand with a pH of 5.5: *A*, Damping-off for consecutive 5-day periods in percent of seedlings exposed, and percent of this total loss caused, respectively, by *Pythium* and *Rhizoctonia; B*, mean daily air and soil temperatures; *C*, total daily precipitation, including water thrown by Skinner irrigation, and mean daily humidity in percent; *D*, daily soil moisture at 8 a. m. expressed as percent of the oven-dry weight of the soil.

the count. Therefore, while the "total" columns in the graphic presentation of the data (fig. 1) represent all seedlings damped-off during the 5-day period, those in the *Pythium* and *Rhizoctonia* columns were secured chiefly from seedlings attacked during the 3 days immediately preceding the count.

EXPERIMENTAL RESULTS

The records of the 1938 studies have been summarized and are presented in graphic form in figure 1. The amount of damping-off is given in the uppermost portion (*A*) while records of temperature (*B*) and moisture (*C* and *D*) appear below in juxtaposition for easy comparison. The amount of damping-off during 5-day intervals is given as the percent of the total number of seedlings in all age classes. Thus on July 9 it appears that, since July 4, 4 percent of all the seedlings in six different age classes (0 to 5, 5 to 10, 10 to 15, 15 to 20, 20 to 25, and 25 to 30 days after emergence) damped-off. Of this 4-percent loss, 80 percent was caused by *Rhizoctonia* and 20 percent by *Pythium*.

Slight variation in age classes appeared in the first two records. On June 9 the only seedlings recorded were those from classes 0 to 5, 5 to 10, and 10 to 15 days after emergence. On June 14, only seedlings from the five age classes 0 to 5, * * *, 20 to 25 days were included. On June 19 and all subsequent dates seedlings from the six age classes 0 to 5, * * * , and 25 to 30 days were included. There was no record for August 23 of the kind of fungi responsible for damping-off.

In general, damping-off increased throughout the summer with rising temperature. A broad survey of the season shows that the sum of damping-off losses recorded for all counting dates in June, each representing total damping-off in all age classes, was approximately 11 percent; in July, 17 percent; and in August, 29 percent. The mean temperatures for the 3 months, respectively, were 67°, 71°, and 73° F. However, there were occasional conflicting fluctuations within the various 5-day periods, such as the decline in amount of disease near the end of July. This decline accompanied and was probably conditioned by cooler weather and dryer soil.

On relative losses caused by *Pythium* and *Rhizoctonia* the influence of temperature and soil moisture was conspicuous. For the season as a whole 54 percent of the total loss was caused by *Pythium* and 46 percent by *Rhizoctonia*. The two organisms were not, however, equally destructive at all times throughout the season, *Rhizoctonia* being the more important after emergence during the spring and *Pythium* during the summer. For the months of June, July, and August *Rhizoctonia* caused 79, 50, and 4 percent, respectively, of the total loss; *Pythium* 21, 50, and 96 percent. Since temperatures increased from month to month, the figures suggest correlations between warm temperature and *Pythium* damping-off after emergence, cooler temperature and *Rhizoctonia* damping-off. The almost complete disappearance of *Rhizoctonia* during and after the hot, dry August weather is conspicuous. Since it operates almost entirely near the surface of the soil, the question arises whether the sun may have had a partially sterilizing effect on the soil surface. *Pythium* commonly operates at lower levels.

A set of means computed for the temperature throughout the season showed that here somewhat raised temperatures favor *Pythium* over *Rhizoctonia.* (See fig. 1, June 24, July 4, July 24, August 3, and August 18.) The range of temperatures, however, was not great—from 74° to 69° F.

Several inconsistencies, nevertheless, did occur. There were severe *Pythium* losses during periods of moderately cool temperature on August 8 and 13, and attacks by *Rhizoctonia* diverged even more from the general correlations of temperature and loss. Severe *Rhizoctonia* losses on July 9 and 19 followed periods of warm temperature while those on June 9, 19, and 29 occurred during cool periods.

Although temperature appeared generally to affect the ratio of *Pythium* to *Rhizoctonia* damping-off, the results for soil moisture were more evident and more consistent. The influence of soil moisture on the relative activity of *Pythium* and *Rhizoctonia* was more clearly shown by examination of figures for the individual 5-day periods than by comparison of the monthly records. As explained earlier, isolations were made from seedlings damped-off only 1 to 3 days before collection. Thus, the material used for isolations July 19 came from plants damped-off during the dry period of July 17, 18, and 19. In this and most other cases (August 3 being a striking exception) *Rhizoctonia* predominated after relatively dry periods and *Pythium* after relatively moist periods. The severe attack by *Pythium* in dry soil on August 3 probably occurred because of the warm temperature during the preceding 3 days. For the seven 5-day periods (isolation periods) during which *Phythium* loss exceeded *Rizoctonia* (fig. 1), the mean soil moisture was 7.2 percent; when *Rhizoctonia* predominated, it was only 5.4 percent.

The 1938 results suggested that while temperature may affect the relative importance of the two fungi, it has a greater influence on total damping-off. Soil moisture, on the other hand, affected more strongly the relative losses caused by the two fungi than it did the total loss. The air-humidity curve in this experiment so closely paralleled that for soil moisture that its influence upon damping-off could not be distinguished.

1939 EXPERIMENTS

The 1938 studies were followed during the summer of 1939 by experiments designed to check more closely on various important points. Improvements made in the 1939 investigations over those in 1938 consisted chiefly (1) of shortening the interval between consecutive counts; (2) of increasing the number of plots so that larger samples of damped-off seedlings would be available for plating and determining the ratio of *Pythium* to *Rhizoctonia*; (3) of maintaining a high soil-moisture content in one of two series of plots to clarify temperature and moisture effects; and (4) of providing plots with different soil reactions.

MATERIALS AND METHODS

The 1939 experiment was conducted on the site used in 1938. Forty 4- by 12-foot seedbeds were placed in 10 rows of 4 beds each between the overhead pipe lines. There were 5 replications and 20 experimental plots, measuring 4 by 4½ feet, that were randomized within each replication. As in the 1938 experiments, these plots represented different seeding dates. However, in 1939 a split-plot design was used in which one-half of each plot was hand-watered daily and the other half given only normal water from rain or the overhead sprinklers. The "normal" half sections were thus identical with the full plots of the 1938 experiment. Inoculation, seeding, and counting of postemergence damping-off were done as in 1938. Similarity of treatment made possible direct comparisons between normal plots during the two seasons as well as between watered and normal plots in the 1939 season.

The watered half-plots were sprinkled daily between 2 and 4 p. m., except on a day of heavy rain or the day following. Water to the equivalent of about 0.2 inch was uniformly applied to each plot.

In taking disease data, figures representing total damping-off and survival, with the plating results from seedlings collected at the different counts, were tabulated as in 1938. Inoculation, seeding, and counting were repeated at 3-day rather than 5-day intervals. Consequently, before the seedlings in any set of plots had stood the 30 days required for relative immunity, they had been counted 10 rather than 6 times.

The weather records in 1939 were kept differently from those shown in figure 1, where the 5-day sums for damping-off were placed parallel to the mean daily weather records. In the 1939 experiments the mean 3-day weather records were calculated and placed opposite the 3-day values for damping-off. Each point on the weather curves in figure 2, therefore, represents the mean of the 3-day period indicated. The bar graphs for rainfall, however, show the totals for the 3-day periods.

The reaction of the soil of the experimental area was changed from about pH 5.5 to 7.0 by thorough mixture of lime with the soil at the rate of 1 ton per acre the fall before seeding. As the season progressed, the acidity drifted to about pH 6.3. This treatment was performed to increase the severity of disease in accord with the opinion of various writers (1, 3, 5, 8) that damping-off is favored by neutral or only slightly acid soils. The results appear in figure 2. During the latter part of July, 1939, an additional companion experiment was set up on soil adjacent to the limed plot. The soil had a reaction of pH 5.5, similar to that employed in 1938. The size and treatments were identical with those of the normal halves of the limed plots. Comparisons showed which of the differences observed between the 1938 and 1939 results were attributable to change in soil reaction and which to seasonal differences. The results are given in figure 3, where the dates and damping-off results at pH 6.3 are the same as those on the right side of figure 2. The corresponding weather data are not repeated, and only the curve for *Rhizoctonia* damping-off is shown. The *Pythium* loss would be represented by a "mirror image" of this curve like that in figure 2, *A*.

EXPERIMENTAL RESULTS

SEASONAL TRENDS

Certain broad seasonal trends will be considered before examining detailed results as influenced by fluctuations in weather. Total damping-off in the normal plots did not differ significantly for the two

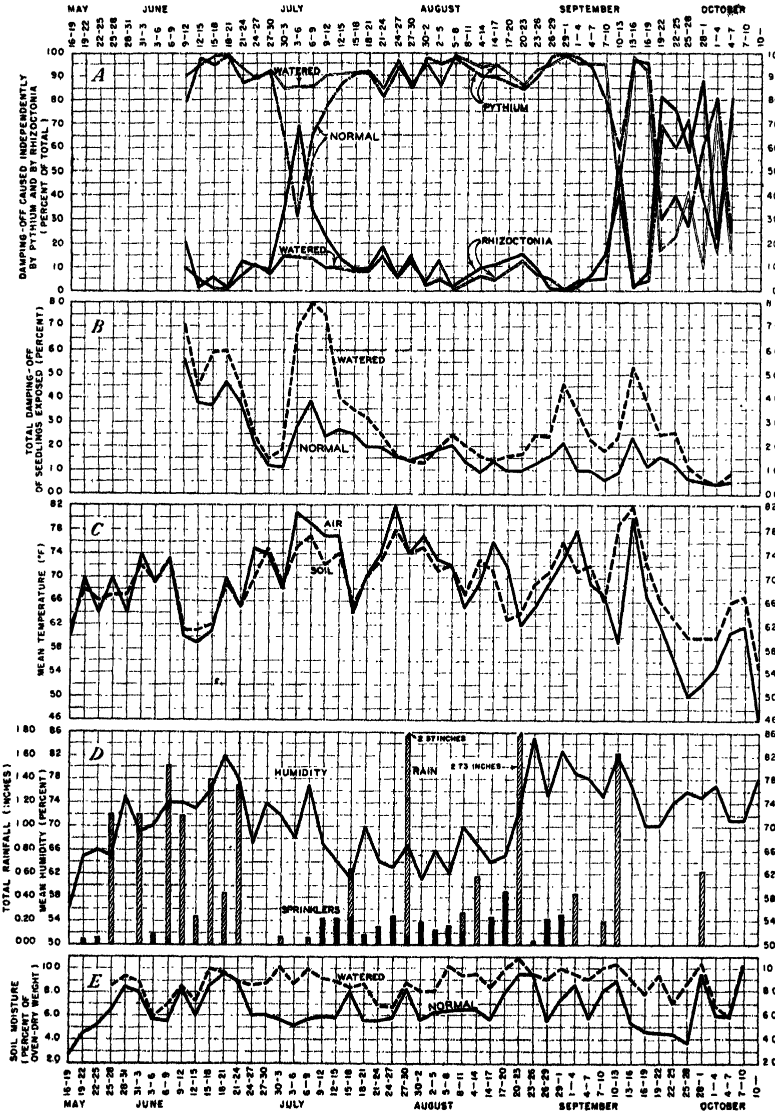

FIGURE 2.—Damping-off of red pine caused, respectively, by *Pythium* and *Rhizoctonia* during the summer of 1939 in inoculated, watered, and "normal" (no special watering) field plots with a pH of 7.0 to 6.3: *A*. Damping-off in watered and normal plots caused independently by *Pythium* and *Rhizoctonia*, expressed as percentage of total loss (thus the loss caused by *Pythium* is the "mirror image" of that by *Rhizoctonia*); *B*, total damping-off for consecutive 3-day periods in percent of seedlings exposed; *C*, mean 3-day air and soil temperatures (° F.); *D*, sum of 3-day precipitation, including water thrown by Skinner irrigation, and mean 3-day humidity in percent; *E*, mean 3-day soil moisture in watered and normal plots expressed as percentage of the oven-dry weight of the soil. Results in more acid (pH5.5) soil appear in figure 3.

seasons. The mean monthly damping-off in 1938 for the period including June, July, and August was 18.9 percent while that for the corresponding period in 1939 was 19.1 percent. The loss in the watered plots, however, in 1939 (30.6 percent) was much greater than in the normal plots during either season. In 1939 the loss was greatest in the spring, becoming progressively less severe as the summer advanced. In 1938, except for the period of light attack late in July, damping-off increased as the summer progressed.

The change in soil reaction appeared not to influence the total loss for the two seasons. Liming, strongly favorable to *Pythium* and

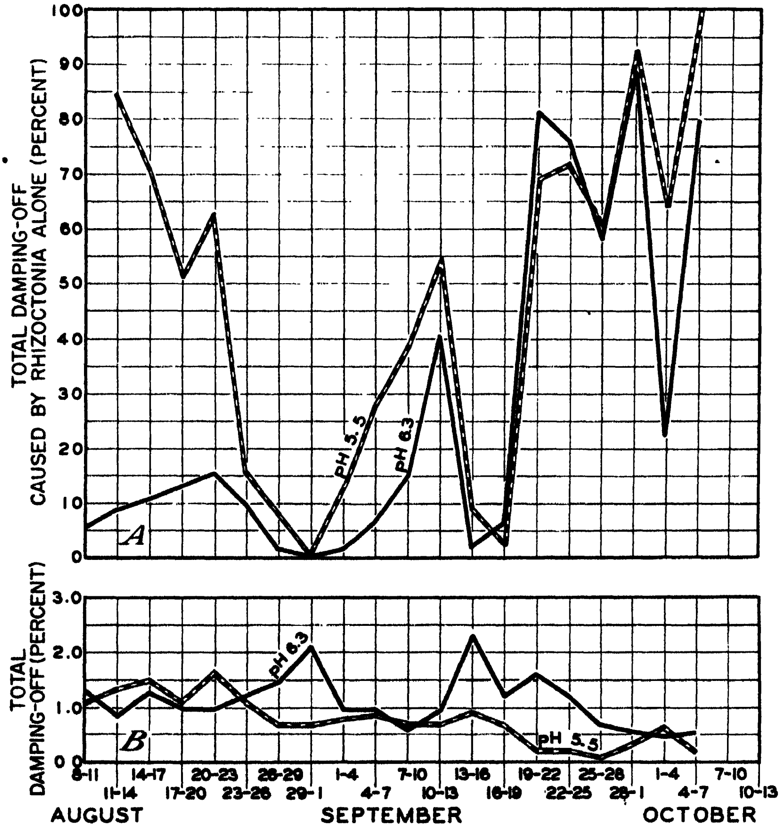

FIGURE 3.—Damping-off of red pine caused by *Pythium* and *Rhizoctonia* between August 8 and October 7, 1939, in inoculated, "natural" field plots in two experiments with respective soil reactions of pH 5.5 and 6.3: *A*, Damping-off caused independently by *Rhizoctonia* expressed as percent of the total loss; *B*, total damping-off for consecutive 3-day periods in percent of seedlings exposed. Other factors influencing the loss were the same as in figure 2 between August 8 and October 7. To avoid confusion of additional lines in *A*, the curves for *Pythium* have been omitted. Since curves for both fungi are based on those in *B*, the *Pythium* curves not shown would be complements of the *Rhizoctonia* curves in *A*. The meteorological conditions are the same as those in figure 2 at corresponding dates.

inhibitory to *Rhizoctonia*, resulted in a reduction of the *Rhizoctonia* loss with a corresponding increase in the number of seedlings killed by *Pythium*. In table 1 are given the relative amounts of damping-off caused independently by the two fungi at different soil reactions and moistures. In normal plots of 1938 with a pH of 5.5, the ratio of losses caused by *Pythium* and *Rhizoctonia*, respectively, was about 1:1. In 1939, after adjustment of the reaction of this same area to pH 7.0, the ratio shifted to about 4:1 in the normal plots and to slightly over 5:1 in the watered plots. During the 2-month period from August 8 to October 7 (the period during which the third experiment was run) the damping-off ratio in the normal plots with a pH of 7.0 was approximately 2.5:1. This value was reduced somewhat from that for the season as a whole because of cool autumn temperatures and low soil moistures which inhibited *Rhizoctonia* less than *Pythium*. However, when the soil reaction was at pH 5.5, return of the ratio to about 1:1 indicated that the differences in the ratios were caused by changes in soil acidity. That temperature and humidity over the ranges encountered were not important in influencing the relative losses caused by *Pythium* and *Rhizoctonia* in the different experiments was suggested by the slight differences in these factors during the two seasons. In 1938 the mean air temperature for the experimental period was 67° F. while that for 1939 was 66°. Humidity during the two seasons was 75 and 74 percent, respectively.

TABLE 1.—*The influence of soil reaction upon the percent of total damping-off caused independently by Pythium and Rhizoctonia in inoculated field plots*

Season and date	Treatment	Soil reaction	Soil moisture	Inoculation made with—	Total damping-off caused by each fungus
1938		*pH*	*Percent*		*Percent*
June 4 to August 24	Normal	5.5	7.1	*Pythium*	53
				Rhizoctonia	47
1939					
June 9 to October 7	Normal	7.0	6.5	*Pythium*	79
				Rhizoctonia	21
	Watered	7.0	8.7	*Pythium*	83
				Rhizoctonia	16
1939					
August 8 to October 7	Normal	7.0	6.6	*Pythium*	72
				Rhizoctonia	28
		5.5	6.6	*Pythium*	51
				Rhizoctonia	49

The effects of temperature noted in 1938 appeared also in 1939. Mean damping-off for the months of June, July, August, and September, respectively, was 3.5, 2.2, 1.5, and 1.2 percent while the corresponding mean air temperatures were 68°, 75°, 70°, and 65° F. Excluding the month of June, there was a progressive decrease in damping-off through the summer with declining temperature. The high damping-off values during June can in large part be attributed to high susceptibility of the seedlings counted during that period rather than to weather conditions alone. All seedlings counted before June 24 (the date at which seedlings of all age classes were included) were approximately 9 days younger in mean age than seedlings counted at all subsequent dates. Their susceptibility was correspondingly greater, as discussed earlier.

The amount of soil moisture in 1939 showed no significant correlation, as in 1938, with the monthly trend of damping-off. However, comparison of the watered and normal plots in 1939 (fig. 2, *B* and *E*) showed that soil moisture greatly influenced total damping-off. In the watered plots, where no dry periods occurred, there was a seasonal mean of 31 percent. In the normal plots a comparable mean was only 19 percent. On only one counting date, August 2, did damping-off in the dry sections exceed the loss in the watered sections of the plots.

These relatively broad observations, dealing with conditions during seasonal or monthly periods, are valuable for indicating trends. However, the actual situations are clarified by studying, through many successive short-time intervals, the changing factors of environment and the corresponding variations in the development of disease.

SHORT-TIME FLUCTUATIONS IN WEATHER

The importance of temperature in the damping-off relationship appears conspicuously in figure 2. It is seen (*B*) in watered plots, provided humidity and soil moisture are high (*E*), that greatest damping-off commonly corresponds with warmest temperatures (*C*). These are conditions of temperature and moisture favoring *Pythium* damping-off, and in view of the neutral soil reaction, a severe loss would be expected. The peaks, e. g., on June 18 to 21, July 6 to 9, and so on, for total damping-off in the normal plots are not so high but correspond with those in the watered plots.

High air humidity and surface moisture (*D*), concurrent with high temperature, correspond with the 3 pronounced peaks of damping-off (*B*) on July 6 to 9, August 29 to September 1, and September 13 to 16. With low humidity the important top one-quarter inch of soil dried rapidly. The humidity was low during those periods of high temperature, July 24 to 27 and August 14 to 17, when damping-off was at a minimum. The relation of high air humidity to damping-off by *Rhizoctonia* has been discussed earlier (*7*).

The influence of soil reaction is seen from a comparison of the results given in figure 3. Total damping-off in the acid soil (pH 5.5) is seen in the latter figure to be sometimes greater and sometimes less than that in the relatively neutral soil (pH 6.3).

Apparently the relative activity of *Pythium* and *Rhizoctonia*, as well as the severity of total damping-off, was influenced especially by soil reaction, temperature, and moisture. In some cases these factors evidently supplemented each other in favoring one of the fungi and discouraging the other.

CHANGING WEATHER AND FUNGUS ACTIVITY

In soils with different acidities (fig. 3) the variations in the amount of damping-off are clarified only when the causal agents and the changing weather (see fig. 2 with corresponding dates) are taken into account. As shown in figure 3, *Rhizoctonia* was more active than *Pythium* in acid soil (pH 5.5) from August 11 to 20, September 10 to 13, and September 19 to October 4. *Pythium* predominated in soil with pH 6.3 as late as September 19 and appeared prominently again October 1 to 4. From August 20 to September 10 and September 13 to 19 temperature and moisture appeared so favorable for *Pythium* that they overbalanced the relatively unfavorable acidity of the soil with pH 5.5.

Temperature and moisture effects are so intimately associated that they are considered together. Their fluctuations influenced the ratio of *Pythium* to *Rhizoctonia* damping-off in both the watered and the normal plots. In the watered plots about 90 percent of the total postemergence loss (fig. 2) was caused by *Pythium* until cool fall weather arrived. From September 10 to 13 the two fungi were about equally active; after September 19 *Rhizoctonia* remained generally predominant against emerged seedlings with the continued low temperatures.

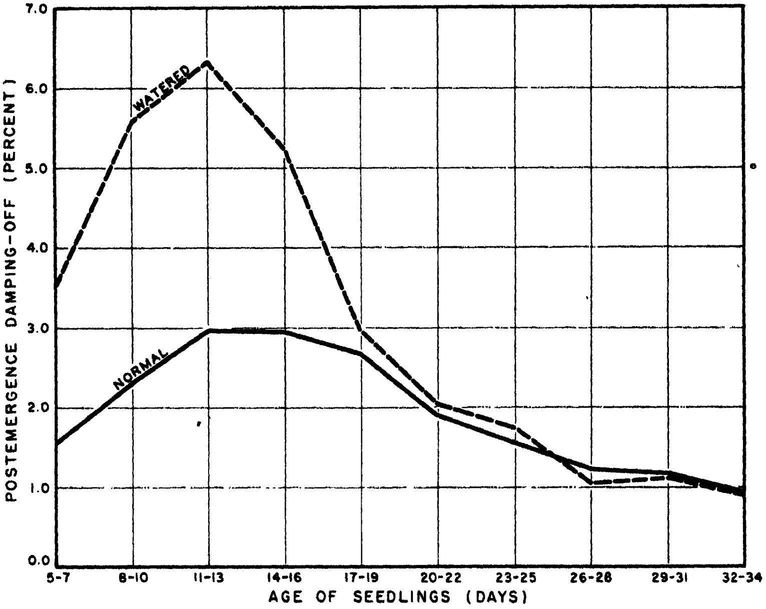

FIGURE 4.—Influence of seedling age on susceptibility of red pine to damping-off by *Pythium* and *Rhizoctonia* inoculated into watered and normal field plots.

In the normal plots the fluctuations in activity by the two fungi were greater than in the watered plots. In general, high soil moisture, high humidity, and warm temperature, when acting together, strongly favored attack by *Pythium* (e. g., fig. 2, June 18 to 21, August 19 to September 4, and September 13 to 16). The inhibition of *Rhizoctonia* activity by abundant soil moisture was indicated by the difference in amount of disease between watered and normal plots and the marked drop after the rain of October 1. However, high air humidity may be associated with severe *Rhizoctonia* attack (fig. 2, July 3 to 9). By encouraging aerial mycelium to grow over the seedlings, it may prompt the most destructive action of this fungus.

In general, it appeared that *Pythium* operated best with relatively wet, not too acid soil and warm temperature while *Rhizoctonia* was more active in relatively acid soil with low moisture and a cool temperature. A single factor or a combination of two or more factors operated at times to inhibit one fungus and to encourage the other.

SEEDLING AGE AND SUSCEPTIBILITY

The varying relation of seedling age to susceptibility is indicated in figure 4. Records were taken at 3-day intervals on the untreated and watered seedlings studied in figure 2. As explained earlier, these had been planted at 5-day intervals throughout the season so that the question of seedling age would not influence the other results. In both watered and normal plots more seedlings damped-off 11 to 13 days after emergence than at any other age. After 13 days in the watered plots there was a sharp decline with increasing age until 17 to 19 days. At greater ages the loss became gradually less pronounced until at 32 to 34 days it fell below 1.0 percent. In the normal plots, after 11 to 13 days, damping-off decreased gradually toward 32 to 34 days. The losses in both normal and watered plots were similar after 3 weeks.

If the seedlings escaped disease for 30 days after emergence, the typical symptoms of damping-off became less and less evident. Although the pathogenic fungi continued to operate under very favorable conditions, the symptoms were commonly those of root rot and seedling blight and hence beyond the scope of this study.

DISCUSSION

The variations in results reported by various authors and mentioned in the introduction have suggested that several factors of unknown influence were involved in the circumstances resulting in the damping-off of red pine seedlings. As explained earlier (*6*), two organisms seemed to be the primary causal agents in Wisconsin. In another study (*7*) temperature, moisture, and soil reaction were observed to influence the activity of both these causal agents. For the most part, within limits, the environment that discouraged one favored the other. This indicates the difficulty of disease prevention by regulating the more obvious environmental factors.

The results of greenhouse studies under controlled conditions correlated closely with results secured in the field under natural conditions. Therefore, the influence of temperature, moisture, and soil acidity, operating in various combinations in the field, could be more exactly interpreted on the basis of the way that it was found to operate in the greenhouse. In making such interpretations, several conditions of study must be explained: (1) In the field studies only postemergence damping-off was considered while taking the records. An estimate of the preemergence damping-off in the field was made and found to correlate closely with that secured in the greenhouse. (2) The time at which the different records were made and averaged for the points indicated in the several figures sometimes caused an appearance of discrepancies. For example, during the period July 27 to 30 there was over 2½ inches of rain. During this period the relative humidity averaged about 66 percent. Obviously, during the heavy rainy period the humidity approximated 100 percent. (3) When meteorological conditions had been unfavorable for the growth of fungi in the soil, fungus attack on the seedlings, as might be expected, lagged for some time after conditions again became favorable. (4) A consideration of weather conditions most favorable for active seedling growth involved such factors as have been explained earlier—a soil reaction between pH 4.5 and 6, soil moisture neither too dry nor too wet, and temperature between 18° and 30° C.

Variations in weather during the critical 30 days following plantings are of primary interest to the nurseryman. The attempt to secure as wide as possible a variation in weather but comparable conditions otherwise presented serious problems. Since variations occur very rapidly within the same season, it was considered that successive plantings throughout two seasons might have a reasonable chance of encountering practically all of the conditions a nurseryman might encounter in the springtime. If one examines the weather records, he finds all variations of temperature and moisture in different combinations, acting at a time when the seedlings are fully susceptible. From the results under a given set of conditions, he may secure a reasonable approximation of what may happen if that kind of weather occurs as his seedlings are emerging.

Most of the discrepancies reported in the introduction have been clarified by showing the presence of two diseases, caused by separate agents, and appearing, respectively, under different environmental conditions. The value of fall planting is consistent with the fact that the seedlings ordinarily emerge very early in the springtime, when temperature suppresses the activity of *Pythium* and high soil moisture represses *Rhizoctonia*. The value of the sulfuric acid soil treatments is apparently increased beyond partial surface disinfection because soil acidity is unfavorable to *Pythium* and soil moisture is often too high for vigorous *Rhizoctonia* development, particularly where the beds are not covered with sterile sand.

Detailed information about the circumstances under which these two fungi operate indicates some requirements for developing control measures.

SUMMARY

The influences of temperature, soil moisture, air humidity, and soil reaction upon damping-off of red pine have been studied in Wisconsin nurseries. The experimental plots were inoculated with local strains of *Pythium* and *Rhizoctonia*, the causal agents of the disease in Wisconsin. The relative importance of the various factors was continually changing with natural variations in the weather. Data in the different experiments were taken at 5- and 3-day intervals throughout two seasons on equal numbers of seedlings. Seed was planted and counts were made at regular time intervals in order that the effects of environmental conditions might be observed on seedlings of comparable ages. Only postemergence damping-off was considered in the records.

The highest percentage of damping-off occurred in seedlings emerged about 11 to 13 days. They were relatively resistant after about 1 month.

The weather often determined which of the two fungi was more active in soil with a reaction between pH 5.5 and 7.0. One or the other might be inhibited, but no natural condition favoring seedling growth inactivated both at the same time.

Variations in soil reaction between pH 5.5 and 7.0 had little influence on total damping-off. A soil reaction of pH 5.5 to 6.0 was favorable to both fungi. More acid soils encouraged *Rhizoctonia* while *Pythium* was favored by soil more nearly neutral.

In general, temperature was more important in determining severity of total damping-off while soil moisture determined which of the two fungi would predominate. Except for early preemergence and some postemergence loss caused by *Pythium* at low temperatures, warm weather favored total damping-off, irrespective of the fungus acting. When temperature was low, postemergence damping-off was at a minimum.

High soil moisture favored *Pythium* while greatest *Rhizoctonia* damage occurred in dry soils. When both fungi were present, the common fluctuations in soil moisture did not greatly affect total damping-off. Warm weather, combined with very high relative humidity, favored rapid aerial growth of *Rhizoctonia*.

An understanding of the conditions favoring or inhibiting the activity of these fungi insures a logical application of control measures.

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SOME CHEMICAL RELATIONS IN THE SUGAR BEET DURING PHASES OF ITS DEVELOPMENT [1]

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INTRODUCTION

Investigations at the Arizona Agricultural Experiment Station on sugar beets (*Beta vulgaris* L.) grown for seed have dealt principally with the physiology of seedstalk induction. The primary objective has been to determine the effect of temperature relations as modified by cultural practices upon vegetative and reproductive development. As a part of the general problem a chemical study of the entire plant was made throughout its life cycle under the climatic conditions which prevail in the Salt River Valley of Arizona and which are typical of the areas of the Southwest where sugar beets are grown for seed by the overwintering method (*6*).[3]

Winter temperatures in many beet-seed-producing areas are near the critical temperature that determines vegetative or reproductive development. In the problem to be discussed in this paper temperature is the chief variable. The lack of specific information on seed-producing plants under field conditions prompted this study of the principal labile carbohydrate and nitrogen fractions in top and root of the sugar beet at intervals during plant development for two successive seasons. Certain chemical relationships revealed by the data are emphasized herein because they suggest interesting phases of the physiology of the beet plant and, possibly, something of the general problem involved in phasic development.

The commercial sugar beet is a biennial, and after a dormant winter period it begins growth anew and forms seedstalk, flowers, and fruit. However, under constant warm greenhouse conditions it will continue vegetative growth indefinitely. Suitable conditions of cool temperature must be experienced by the plant to induce the reproductive phase. Sugar-beet plants differ greatly in their tendency to bolt, that is, to form seedstalks. Plants that respond to relatively slight exposure to cold are usually referred to as easy or fast bolters, while those that require relatively great exposure to cool temperature are hard or slow bolters. Plants grown for seed are not thinned, in contrast to the practice of thinning beets grown for sugar production. Early plantings made in August have a longer period of vigorous growth during the autumn than later plantings, and the larger foliar cover shades the soil more completely and reduces the surface soil temperature. Thin stands of plants reduce this cooling effect.

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[2] Valuable advice given by Dr. Eubanks Carsner during the course of these experiments is gratefully acknowledged.

[3] Italic numbers in parentheses refer to Literature Cited, p. 445.

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Both vegetative and reproductive sugar-beet plants produce large storage roots. Typical differences in their shape, size, and sugar content have been discussed by Esau (*2*). In this study the roots of bolting plants were smaller and attained a higher sucrose concentration than the roots of typically vegetative plants, as grown for sugar, but because of their greater size, the vegetative plants contained greater absolute amounts of sucrose per plant. The leaf area was approximately equal in vegetative and reproductive plants of the same age.

The general effect of temperature upon reproduction in the sugar beet is well known. Chroboczek (*1*) and Roberts and Struckmeyer (*10*) found it to be a long-day, cool temperature plant. Owen, Carsner, and Stout (7) showed that the relative effects of temperature and day length vary with the genetic constitution of the plant. For biennial beets they found that the temperature effect was predominant, but also found evidence of the production of flower-inducing substances by the plant. Information on the chemical behavior of sugar beets has been provided by the work of Pultz (*9*) at St. George, Utah, on a variety of easy-bolting beets grown under conditions of temperature favorable to bolting, in which the chief variable was the nitrogen supply of the soil. It was shown by Pultz that sucrose and nitrogen were withdrawn from the root during seedstalk development. When nitrogen was limiting, sucrose accumulated, flowering was suppressed, and the seed yield was lowered.

EXPERIMENTAL DETAILS

Sugar beets were grown in plots near Mesa, Ariz., within the seed-producing area of the Salt River Valley. In the first year's experiments six varieties[4] of sugar beets varying in characteristics with respect to disease resistance and bolting tendency were grown in duplicate plots of approximately 0.1 acre per variety for each of three planting dates (August 16, September 17, and October 2). The plants were grown on soil judged from past records to be of high and uniform fertility. Ten tons of barnyard manure per acre was applied before the soil was prepared. Ammonium phosphate was applied at the time of seeding. Seed was sown at the rate of 18 pounds per acre in rows 20 inches apart on two-row beds centered 24 inches apart. Additional nitrate was added March 7. It is believed that no nutritional factor was limiting, except possibly nitrogen for a short period during the first winter in the August and September plantings. Differences in plant responses are assumed to reflect the effects of temperature. Cultural practices incident to date of planting, extent of shading, spacing of plants, and irrigation provided a differential of temperature whereby the soil at the crown of the beets of the August planting was several degrees cooler than that of the October planting during several hours each day for most of the growing season, as shown by thermograph records (fig. 1). A sample for a chemical test was a composite of all varieties for each planting date. Determinations for reducing sugars, total sugars, amino nitrogen, total nitrogen, nitrate nitrogen, and amide nitrogen were made November 25, December 29, March 2, April 13, and June 3. Soil nitrate was determined for each foot increment to a depth of 5 feet for each plot on each of these dates. The soil was Laveen loam, characterized as follows:[5]

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[4] The varieties grown were U. S. 12, U. S. 14, U. S. 33, U. S. 217, Great Western, and Rabbethge & Giesecke (old type).

Total soluble salts, 1,200 p. p. m. of dry soil
  pH at moisture equivalent, 7.7
  at 1:10 dilution, 8.8
Neubauer values:
  23 mg. K per 100 gm. of soil
  5.2 mg. PO_4 per 100 gm. of soil

Experiments for the second season were limited to 1 slow-bolting variety planted in duplicate plots of approximately 0.3 acre per plot for each date of planting. Five of the 32 rows in 1 plot of each planting date were thinned to 1 plant per foot to produce a slight difference in soil temperature between plants of the same date of planting, but otherwise environmental conditions were the same. Chemical determinations were made upon both thinned and unthinned plants of the August and October plantings and also upon the unthinned September plants. Thus each season 3 plantings were made, designed to produce different degrees of thermal induction of the reproductive phase. The relatively cold winter of the first season favored this induction and a high seed yield except in plants at the extreme south end of the plots where the soil was subjected to little shading and no induction occurred. These latter plants were used as typical nonbolters. In the second season three degrees of thermal induction were provided by date of planting as before, and in addition a more precise study of slight temperature differences was provided by spacing of the plants. The chance of variability as a factor was reduced by limiting the study to 1 variety. Average temperature for the second season was less favorable for induction of the reproductive phase, thus placing all plantings of that season very near the critical temperature relationship.

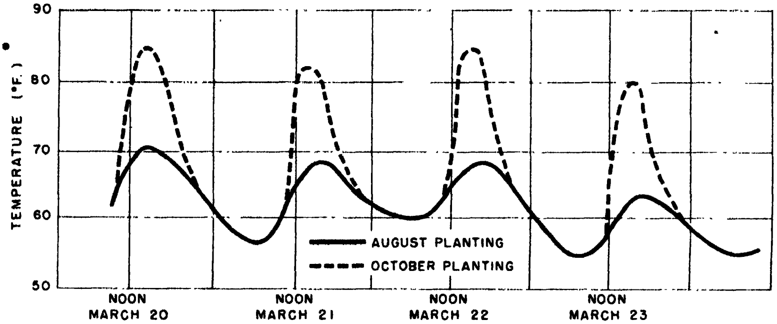

FIGURE 1.—Soil temperature at crown of beets during March 1939.

CHEMICAL METHODS

A relatively large amount of material was gathered from each plot and rushed to the laboratory under cool, moist conditions where representative samples of sap and tissue were prepared and refrigerated. Amino acids were determined at once on the sap by the

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[5] Data furnished by Dr. T. F. Buehrer of the Arizona Agricultural Experiment Station.

Van Slyke method (*11*), amides were promptly hydrolyzed and their determination completed shortly thereafter by the Vickery-Pucher method (*12*), sugars were determined from samples of the expressed sap by the ceric sulphate method described by Hassid (*4*), and nitrate by the Devarda method. Total nitrogen was found by the Kjeldahl method on finely ground tissue in which the nitrate had been reduced by iron (*8*). Soil nitrate was ascertained by the phenoldisulfonic method.

RESULTS

Solutes are reported in terms of concentration on the assumption that concentration has greater physiological significance than dry-weight relations. Figure 2 illustrates the general behavior of reducing sugars as shown by the seed-producing plants for 1938–39. In the top tissue there was an increase in the concentration during early development, followed by a decline during seed development. The root tissue was characterized by a very low concentration of reducing sugars throughout the season. A marked difference existed between root and top concentration, but this difference was characteristic of both vegetative and reproductive plants. The second season showed the same general trend except that no significant decline in concentration of reducing sugar occurred in the top tissue during the reproductive stage. The seed yield for the second season was quite low.

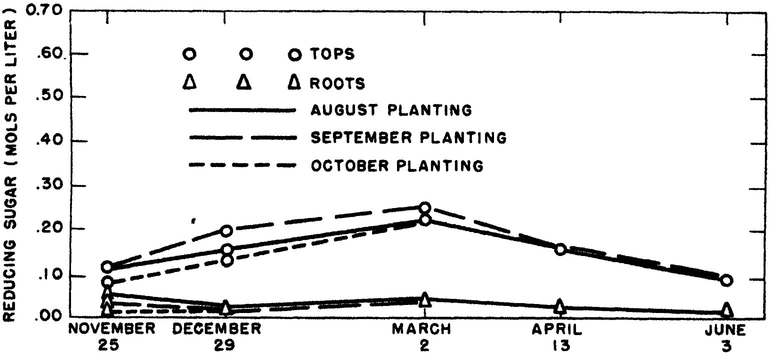

FIGURE 2.—Reducing sugars in sugar beets during development, 1938–39.

The concentration of sucrose in plants for 1938–39 is shown in figure 3. The early plantings (August and September) rapidly built up the sucrose concentration to high levels until bolting time, after which it rapidly declined. These plants produced heavy seed yields. The October planting maintained a lower sucrose level during its vegetative development, but continued to increase the concentration even during the bolting period, and markedly so during the period of seed development to the extent that on June 3 the late (October) planting had a decidedly higher concentration of sucrose than the early plantings. The absolute amount of sucrose per plant was much less in the late than in the early plantings. The October planting yielded less than one-half the seed produced by either of the earlier plantings.

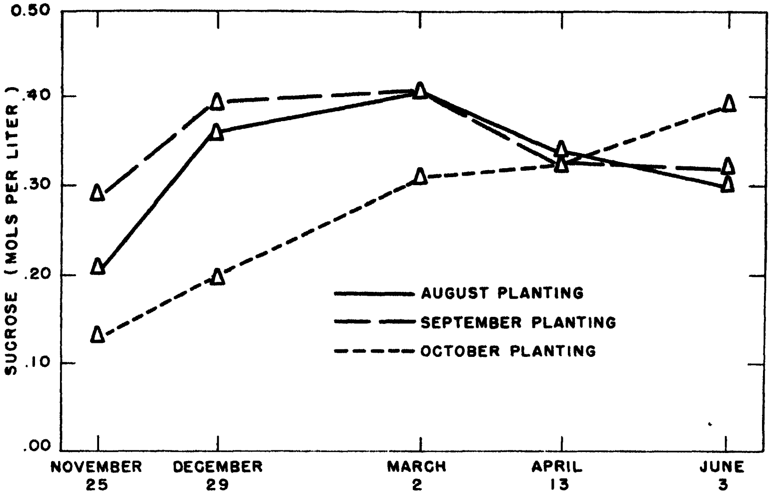

FIGURE 3.—Sucrose in sugar-beet roots during development, 1938–39.

Figure 4 indicates the trends of sucrose concentration observed during the season 1939–40. August and October plantings each have parallel experiments, namely, with thinned and unthinned plants. All plantings show a continuous increase of sucrose concentration in

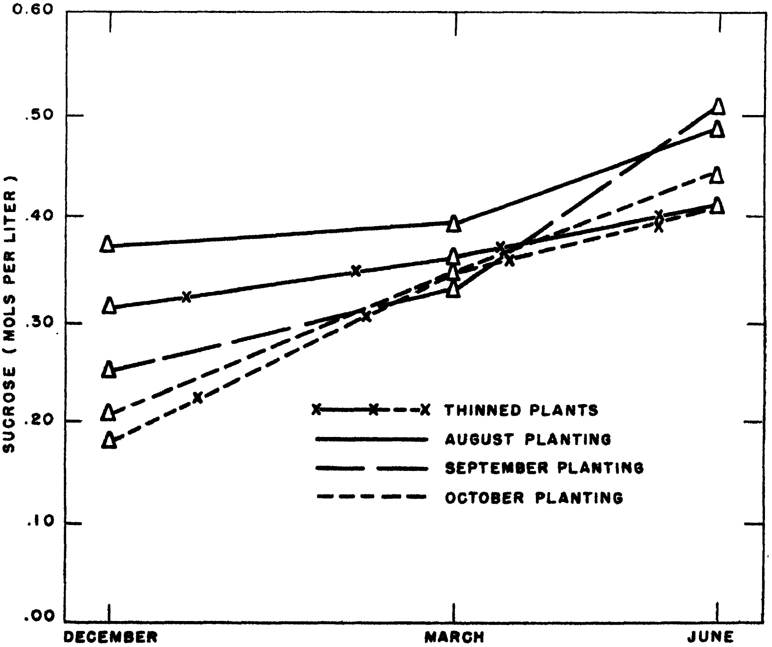

FIGURE 4.—Sucrose in sugar-beet roots during development, 1939–40.

the root tissue throughout the season. The early plantings (August and September) again showed the higher concentration in the early stage of development, but by June 5 the late planting (October) approached the same level of sucrose as the earlier plantings. During early development the thinned plants in both August and October plantings maintained a lower sucrose concentration than the unthinned plants. The thinned plants of these two widely divergent planting dates both showed a decline in the rate of sucrose accumulation during the bolting season and both attained approximately the same level of sucrose concentration by June 5. The thinned October planting produced no seed and all other plantings gave very poor seed yields.

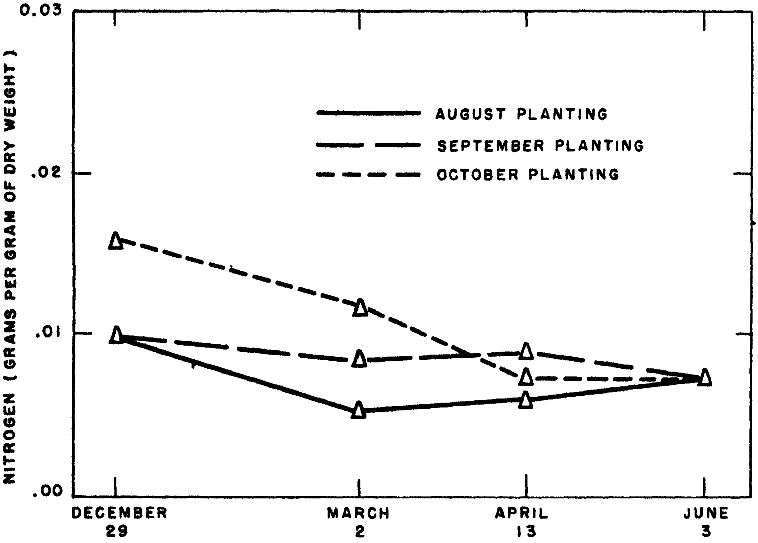

Figure 5.—Total nitrogen in sugar-beet roots during development, 1938–39.

The seasonal trend of total nitrogen in root tissue for 1938–39 is indicated in figure 5. A decline in the percentage of total nitrogen for all reproductive plants is characteristic. When data for these plants are compared with those for nonbolting plants (table 1) it appears that relatively large absolute amounts of nitrogen have disappeared from the roots during reproductive development. The major loss apparently takes place about the time of seedstalk development.

In figure 6 is shown the trend of total nitrogen in August and October plantings for the following season (1939–40). The unthinned plants show a marked decline during the bolting period. The thinned plants show a distinctly different behavior with respect to total nitrogen. The thinned August planting bolted and produced some seed; the thinned October planting produced no seed. Both thinned plantings were relatively high in total nitrogen while both unthinned plantings produced more seed and lost total nitrogen from the root tissue.

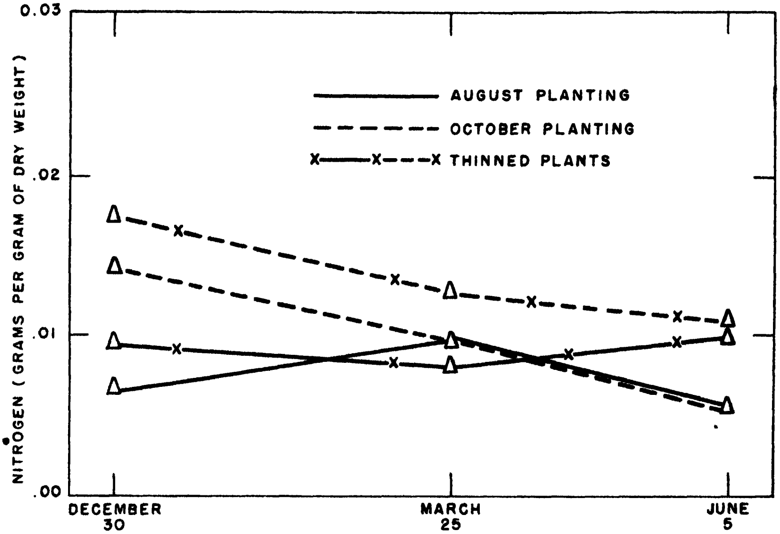

FIGURE 6.—Total nitrogen in sugar-beet roots during development, 1939–40.

TABLE 1.—*Comparison of carbohydrate and nitrogen fractions in bolting and nonbolting sugar-beet plants at harvesttime*

Fraction	Tissue	Planting date			
		Bolters		Nonbolters	
		Aug. 16	Sept. 7	Oct. 2	Sept. 7
Reducing sugars ..... mols per liter	Tops	0.071	0.078	0.078	0.055
	Roots	.006	.007	.006	.009
Sucrose ..... do	Tops	.000	.006	.005	.002
	Roots	.324	.341	.387	.315
Nitrate nitrogen ..... do	Tops	.0083	.0087	.0013	.0478
	Roots	.0039	.0071	.0066	.0636
Amide nitrogen ..... do	Tops	.0036	.0019	.0073	.0062
	Roots	.0013	.0013	.0013	.0096
Amino nitrogen ..... do	Tops	.0220	.0173	.0188	.0519
	Roots	.0162	.0229	.0298	.1163
Total nitrogen per gram of dry weight grams	Tops	.0441	.0273	.0316	.0318
	Roots	.0068	.0070	.0070	.0155

Nitrate nitrogen represents from 3 to 11 percent of the total nitrogen found in the plant. The nitrate relationships are illustrated in figures 7, 8, 9, and 10. The trend of nitrate nitrogen in the tops of the 1938–39 plants, which is shown in figure 7, is characterized by a marked reduction in concentration during the reproductive phase. A similar trend in the root tissue is indicated in figure 8. Figure 9 shows the behavior of the plants in the parallel experiments in the August planting of the 1939–40 season. The unthinned plants show the same decrease in nitrate concentration noted before, but the thinned plants show little change in concentration in either top or root tissue during the period of flowering and seed development. Similar trends are apparent in the October planting (fig. 10). In all cases except the October planting of 1938–39 the unthinned seed-producing plants

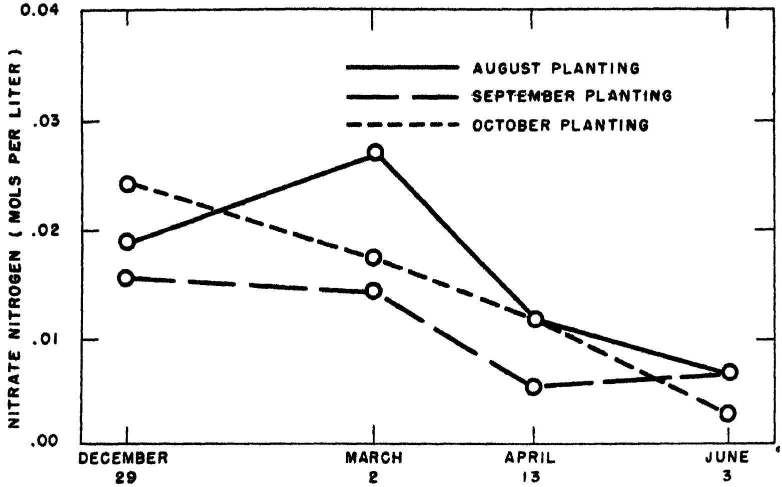

FIGURE 7.—Nitrate nitrogen in sugar-beet tops during development, 1938–39.

developed a nitrate relationship such that the concentration was relatively much greater in the tops than in the roots. In the parallel experiments the thinned plants either reversed this relationship or showed a strong tendency to do so. The concentration of nitrate in the tissues of the plant was always manyfold greater than the concentration in the soil (figs. 9 and 10).

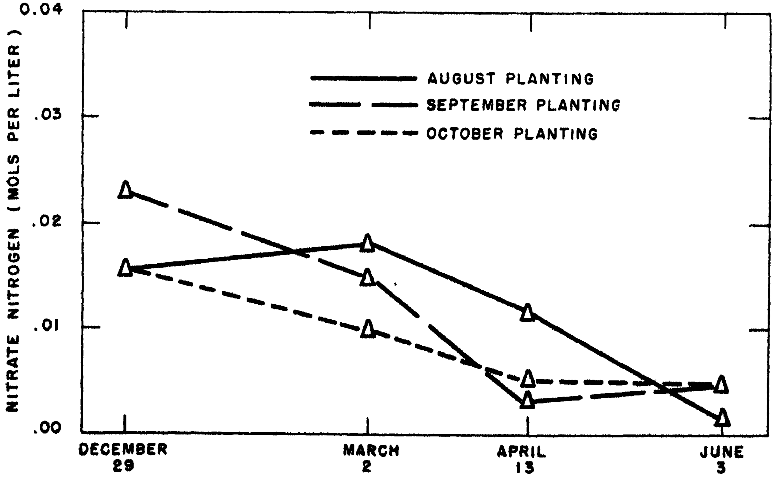

FIGURE 8.—Nitrate nitrogen in sugar-beet roots during development, 1938–39.

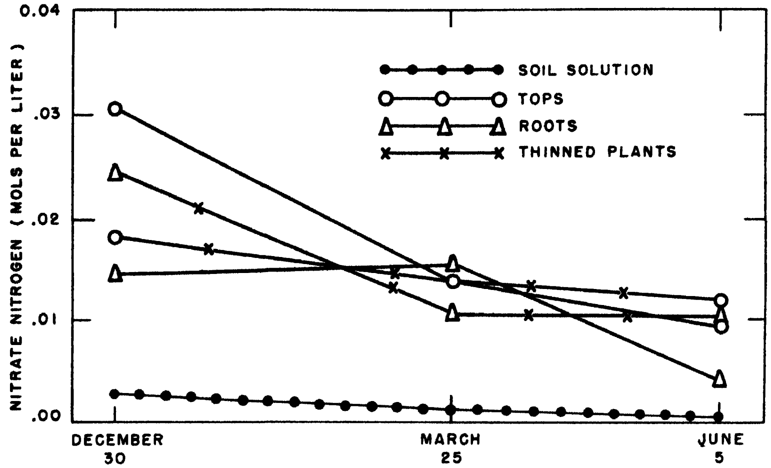

FIGURE 9.—Nitrate nitrogen in sugar beets (August planting) during development, 1939-40.

The dominance of the vegetative or reproductive phase of development during the season is illustrated by the relative weight relations between top and root for the first season's plantings as shown in table 2. The degree of successful reproduction attained by the

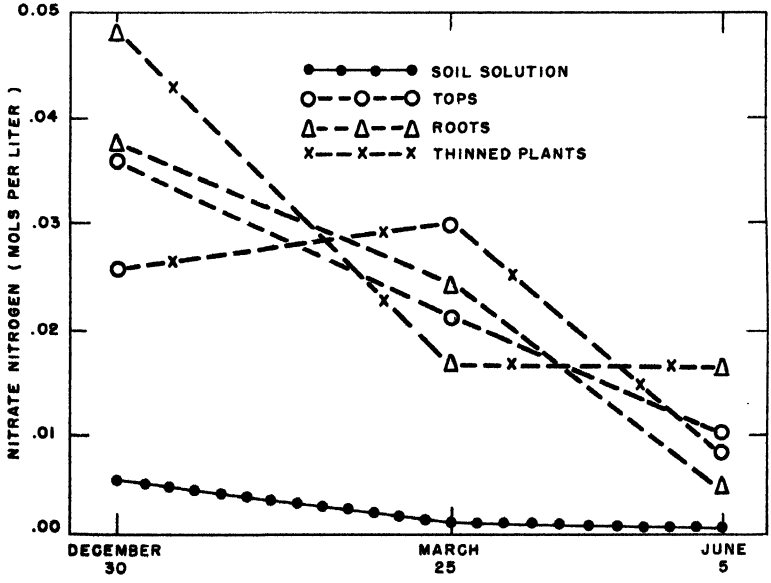

FIGURE 10.—Nitrate nitrogen in sugar beets (October planting), during development, 1939–40.

TABLE 2.—*Top-root weight ratios of sugar-beet plants grown for seed, 1938–39*

Planting date	Date of sampling			
	Nov. 25	Dec. 29	Mar. 2	Apr. 13
Aug. 16	1.63	1.76	1.19	1.68
Sept. 7	4.38	2.97	1.81	2.76
Oct. 2	6.85	3.47	2.10	1.57

various plantings may be estimated from the seed yields given in table 3.

TABLE 3.—*Seed yields*[1] *from a variety of sugar beets low in bolting tendency*

Planting date	Yield per acre	Planting date	Yield per acre
1938	*Pounds*	*1939*	*Pounds*
Aug. 16	2,168	Aug. 23	580
Sept. 7	2,545	Sept. 16	321
Oct. 2	917	Oct. 7	179

[1] No records made of yields from thinned plots; no seed produced by thinned plot of Oct. 7, 1939.

DISCUSSION

Both the vegetative and reproductive phases of development in the sugar beet are favored by an abundant supply of nitrogen (*9*). Since the sugar beet is a long-day plant the induction of the reproductive phase should cause neither check in growth nor accumulation of reserves as has been reported for short-day plants (*5*). Therefore, characteristic trends in chemical relations shown by the data on sugar beets appear to have more than ordinary significance. Trends which appear important here are the carbohydrate, total nitrogen, and nitrate nitrogen relationships during the vegetative and reproductive phases of the plant. The need for discernment of various stages in the reproductive phase brought about by either photo induction or thermal induction has been stressed by Hamner (*3*). For the sugar-beet plant attention may be focused upon those plants that are strictly vegetative, those that bolt but produce little seed, and those that produce heavy seed yields.

Reducing sugars throughout the plant were generally of the same order in corresponding parts of plants of the same age and their concentration and movement did not seem to be a limiting factor except in the October planting for 1938–39 where the low concentration in the tops conceivably could have been responsible for low seed yields. The decline in reducing sugars wherever heavy seed production was in process suggests that reducing sugars constitute an important step in these transformations.

Sucrose appears to be the principal source of mobile carbohydrates, supplemented by the daily contribution by photosynthesis. Plants that produce heavy seed yields draw heavily upon the sucrose in the root. Such utilization involves a hydrolytic process which did not appear to be limiting except, possibly, in the case noted above. Plants that bolt but make poor seed yields continue to increase the sucrose concentration in the root tissue during the bolting period. Plants of the same age that remain strictly vegetative likewise continue to accumulate sucrose. The tendency to store or utilize sucrose apparently becomes a measure of vegetative or reproductive tendencies in the beet plant.

During the reproductive phase the percentage of total nitrogen decreased more rapidly than the rate of growth of the root, about 50 percent of the nitrogen actually disappearing from the root. In the vegetative plant of the same age the percentage of total nitrogen increased during the same period. Since only a small percentage of the nitrogen was soluble at any given time a hydrolytic process was involved in the reproductive phase. The tendency to export nitrogen from the root may be considered a reproductive characteristic of the sugar beet.

So far as may be judged from the results of this study, the nitrate nitrogen appears to be the most mobile nitrogen fraction. At all times the nitrate concentration was manyfold greater throughout the plant than in the soil solution. The equilibrium concentration (or steady state) near seed harvesttime was markedly different in plants of the same age but in different stages of development. In strictly vegetative plants the nitrate concentration was severalfold greater than in reproductive plants. It had an intermediate value in wealky reproductive plants. In vegetative plants the concentration in the root exceeded the concentration in the top as if the nitrate distribution depended upon a concentration gradient from the root. In typically reproductive plants the nitrate equilibrium maintained a higher concentration in the top as if the nitrate were transported through the plant by the utilization of energy, as has been postulated for other ions. The tendency for nitrate to accumulate in the tops of reproductive plants has been observed also by Dr. J. M. Fife[6] in four successive seasons. Unthinned plants from the parallel experiments of the second season followed the pattern for reproductive plants, while the thinned plants, subjected to slightly higher soil temperatures showed the nitrate relationship characteristic of vegetative plants. The low-yielding plants (October) of 1938–39 that showed strong vegetative characteristics with regard to sucrose accumulation and top-root weight ratio also had a nitrate relationship characteristic of vegetative plants.

In those cases where the nitrogen supply and temperature were both favorable the plants utilized nitrogen and sucrose from the root, a finding which is in agreement with previously reported observations of Pultz (*9*). When the temperature relation was unfavorable for reproduction this fact was reflected in the chemical relations within the plant and nitrogen was not used freely for flower and seed even in the presence of an ample supply of nitrogen in the plant tissue. Seed yields and temperature records for these experiments fully support the conclusions of Owen, Carsner, and Stout (*7*) on the relation of temperature to bolting. Differences in certain chemical relations observed in plants that experienced different temperature exposures may be interpreted as supporting the concept of the induction of substances essential to the reproductive phase through temperature relations. However, the nature of the special agents responsible for the observed chemical relations is conjectural.

The foregoing observations show that the loss of sucrose from the root is roughly proportional to the seed production, the loss of nitrogen is greatest during seedstalk formation, and the most striking changes in nitrate concentration and top-root nitrate ratio occur during the period of seed maturation. There appears to be fairly good evidence for a correlation of the storage of sucrose in the root, high total nitrogen, high nitrate concentration throughout the plant, a top-root nitrate ratio less than unity, and a decreasing top-root weight ratio with the vegetative phase. On the other hand, the loss of sucrose and nitrogen from the root, a declining nitrate concentration, a high top-root nitrate ratio, and a high top-root weight ratio are charcateristic of the reproductive phase. The change in equilibrium for any of these systems appears to be associated with a certain stage of development. The extent to which these relationships between development and chemical composition are causal or resultant is debatable. The more important consideration is the agency that directs the chemical reactions toward one or the other phase of development.

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[6] Correspondence.

Ordinarily each of the important processes that have been discussed is considered to be controlled rigidly by one or more enzyme systems. The agents necessary to the initiation of these processes are produced, by this plant under favorable conditions of temperature. The temperature requirements for their production differ significantly and the degree to which any of the chemical relationships is attained in the plant depends upon thermal relationships. It is not clear whether a group of enzyme systems acting independently performs the role sometimes attributed to a flowering hormone or whether the operation of these systems depends upon the activity of one or more hormone-like substances. The need for a better understanding of enzyme systems in plant development is evident. The data indicate that a number of processes, such as the hydrolyses of sucrose and insoluble forms of nitrogen, the special distribution of nitrate, reduction of nitrate and syntheses of certain compounds, are involved in reproduction in the sugar beet and that the coordination of all these processes is essential to successful reproduction. It appears that a consideration of the larger chemical fractions as found in suitable plants grown under appropriate conditions will contribute much toward the analysis of the problems of phasic development.

SUMMARY

Sugar beets were grown for seed in the Salt River Valley of Arizona under favorable nutritional conditions. Temperature relations were varied through cultural practices. Chemical determinations were made on important carbohydrate and nitrogen fractions in tops and roots throughout the vegetative and reproductive phases for two successive seasons.

Comparison of these chemical fractions, as found in strongly vegetative plants and in plants subjected to various degrees of induction to the reproductive phase, has been made. Seed yield was used as a criterion of successful reproduction.

Vegetative plants stored sucrose and nitrogen in the roots. Nitrate attained a relatively high concentration throughout the plant, with the root concentration in excess of the top.

High seed-yielding plants utilized sucrose and total nitrogen stored in the root. Nitrate showed marked reduction in concentration during the reproductive phase, characterized by a higher concentration in top than root.

Low seed-yielding plants utilized less than the daily supply of sugars provided by photosynthesis and stored sucrose during the bolting period. Nitrogen relations within the plant reflected the degree of induction to the reproductive phase brought about by temperature.

Some possible relationships between chemical composition and phasic development have been discussed.

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INHERITANCE OF SIZE IN SINGLE-COMB WHITE LEGHORNS[1]

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INTRODUCTION

Investigators who have studied the inheritance of body size have employed a variety of methods, the most common of which involve the crossing of animals of divergent sizes. The mean size and variability of the F_1, subsequent generations, and backcrosses have led most workers to conclude that body size is under polygenic control. Usually animals of different breeds or varieties of a given species or even animals of different species have been used in such studies, since the existing interbreed and interspecific variability provides a wide range of characteristic body sizes. This is especially true of the domestic animals in which generations of artificial selection have resulted in the establishment of breeds differing in size by several hundred percent.

Instances of crosses between breeds of poultry of such extreme divergence are furnished by the studies of Punnett and Bailey (*18*),[2] Jull and Quinn (*8*), and Maw (*16*), all of whom used Bantams as one of the parents. As noted, inheritance on a polygenic basis was indicated in these cases, as well as in crosses between breeds less dissimilar in size, including those made by authors tending to interpret the F_1 and other segregations on the assumption of a limited number of genes (cf. the two-gene hypothesis of Waters (*22*) or the "four or more" pairs of genes suggested by Quisenberry, Roberts, and Card (*19*). Interpretation on a polygenic basis also appeared to satisfy the results of studies conducted on crosses in other species than chickens, e. g., ducks (*17*, *4*), pigeons (*23*), and most of the commonly studied laboratory mammals. (See Lerner (*9*) for a review.)

The variation in mature size within breeds has been studied far less intensively. In chickens Lerner (*10*) ,using shank length as a criterion of size, found two strains of Single-Comb White Leghorns to exhibit significant differences, with the F_1 and backcrosses intermediate in size. Earlier Dunn (*3*) reported similar differences in the length of the long bones between inbred families of the same breed. Of two crosses between such families one F_1 was intermediate and the other exceeded both parents.

Other investigations on the genetic basis of size differences within breeds include a limited number of statistical studies on Jersey cattle and swine. In the former Gowen (*7*) noted that some 60 percent of the variance in size was hereditary. In swine the genetic portion of the variance was found to rise from an insignificant figure at birth (*14*) to 18 percent at weaning time (*2*), and to 30 to 40+ percent at 180

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[2] Italic numbers in parentheses refer to Literature Cited, p. 456.

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days of age (*24*). The increase of the genetic portion of the variance with age reflects the existence of significant nongenetic effects of nutrition during the embryonic and the nursing stages of mammals (at weaning time 40 percent of the total variance was due to environment common to litter mates). Such effects are likely to be of much smaller importance at comparable ages of birds.

All in all, it may be seen from the above review of literature that the information on the extent of the hereditary control of adult size within breeds is very limited. The present study was designed to throw some light on this subject, with the Leghorn breed of chickens used as experimental material.

MATERIAL AND METHODS

Body size may be expressed in terms of body weight or in terms of some skeletal measurement. The latter is preferable since body weight is subject to a great deal of fluctuation due to nutritional state, condition of the ovary, and other factors of internal and external environment. Mature length of shank, a measurement bearing a close relation to the actual length of the tarsometatarsus ($r = 0.968 \pm 0.007$ with a standard error of estimate of 0.028 cm. (*1*) has been found to be of use in expressing size differences. Its correlation with body weight is of the magnitude of 0.66 (*10*), so that not all of the variation in body weight is accounted for by shank-length differences between birds. For the purposes of the present study mature shank length is used as a criterion of size but with the understanding that it does not necessarily represent the same genetic differentials that would be manifested by the variation of body weight.

Measurements of shank length were made on mature females from the University of California flock with the device described by Burmester and Lerner (*1*). The production-bred strain of Leghorns with which the experiment originated consisted of approximately 450 to 650 pullets hatched annually in March and April. These were selected for high production and viability since 1933 with some introduction of stock from private breeders. Of particular importance in connection with this experiment is the extensive use made in breeding of the offspring of 3 males and 1 female from the strain developed by the Kimber Breeding Farms at Niles, Calif. An earlier report (*10*) gave detailed information on the differences in size between the Kimber strain and the University of California flock. The mature females of the Kimber strain averaged 10.28 cm. in shank length as compared with a mean of 9.59 cm. for the University flock.

In 1938 when the present experiment was started, a considerable proportion of the University flock had one or more of the four Kimber birds represented in their pedigrees. In December of that year the first measurements relating to this study were made. All full-sister families of five or more pullets originating from sires with a minimum of three such families were measured. In subsequent years the same procedure was followed, but the bases of selection of breeding birds to perpetuate the production line did not take these measurements into consideration. The production line can thus be considered as a control population, random-bred with respect to size. The exception to this statement lies in the fact that the foundation stock for the large size line was made ineligible to serve as breeders for the production line. In this manner a minor degree of selection against large size was exercised in these quasi controls.

The size line of 1939 originated from two males and eight dams. In the following years several dams not previously represented in the size line were introduced from the production line (four in 1940, and two in 1941). In the size selection all of the female offspring alive in December of their year of hatch were measured irrespective of the number of sisters in the family.

The number of birds measured in each year in each line and the mean shank measurements appear in table 1 under the heading "Total population." For the purposes of statistical analyses in a subsequent section those families which answered the requirements of numbers as outlined for the production line were selected. The mean shank measurements for these birds also appear in table 1 under the heading "Restricted population." The discrepancies between the number of birds in the two populations of the production line are due to the fact that in some cases measurements on all members of a family were not obtained, thus reducing the size of the family below the minimum number set. Failure to obtain measurements on these birds was usually due to bumblefoot, an abscess on the ball of the foot, which prevented the application of the measuring device.

TABLE 1.—*Mean shank length for birds measured in each year in each line*

Year	Total population				Restricted population			
	Size line		Production line		Size line		Production line	
	Birds measured	Mean shank length	Birds measured	Mean shank length	Birds measured	Mean shank length	Birds measured	Mean shank length
	Number	*Centimeters*	*Number*	*Centimeters*	*Number*	*Centimeters*	*Number*	*Centimeters*
1938	----------	----------	368	9.69	----------	----------	368	9.69
1939	84	9.92	274	9.62	84	9.92	240	9.65
1940	83	9.94	137	9.55	59	9.89	121	9.59
1941	106	10.20	346	9.37	61	10.18	346	9.37
1942	119	10.29	260	9.46	86	10.30	256	9.46

The selection of breeding birds in the size line was based on progeny- and sister-test data, and, within families of full sisters, on the phenotype. Some effort was made to maintain in the size line the high egg-production characters of the production line, with particular emphasis on early sexual maturity. In order to maintain the economic qualities of the size line late-maturing birds (over 200 days of age at their first egg) were discriminated against in the selection of breeders. In spite of this the 1942 size line matured significantly later than the corresponding production line by 10.7±3.0 days (176.9 as against 166.2 days).

Males were selected entirely on the basis of progeny and sister tests. It has not been economically possible to keep all the males till maturity, and the information on the relation of early to mature shank length in these lines has not been sufficient to base the selection of males on their shank length at 6 weeks of age (the time when most males are culled in this flock).

It should be noted that the small increase in the mean shank length of the fourth selected generation over that of the third (0.09 cm.) does not necessarily indicate an approach to a plateau. It so happens that one of the three sires used in 1942 had progeny averaging lower than the mean of the 1941 population (10.06 vs. 10.20 cm.). The number of offspring from the other two sires (averaging 10.36 and 10.43 cm. respectively) was not sufficiently large to compensate entirely for the choice of this one male.

As noted previously, the selection of breeders in the production line was independent of the measurements made. The amount of inbreeding in this line did not show any rapid increase in the experimental years. As an indication of this, the average coefficients of inbreeding of the sires of the restricted population were for the years 1938 to 1942, respectively, 4.13, 7.26, 5.94, 5.25, and 2.93 percent. Similar figures for the sires of the size line (restricted poulation for the years 1939 to 1942) were 3.52, 7.55, 2.35, and 11.81 percent. A better indication of the increasing homozygosity of the size line is given by the inbreeding coefficients of the extreme variant families of sisters (with the longest shanks) in that line for each year from 1939 to 1942, which were respectively 3.13, 4.69, 7.47, and 15.95 percent.

RESULTS OF SELECTION

As table 1 shows, the four generations of selection have undoubtedly been successful in increasing the shank length of the size line and differentiating it from the production line.

There are at least two possible factors responsible for lack of even greater progress: (1) The small scale of breeding operations, and (2) the damping effect on selection for size exercised by the discrimination against late-maturing birds. A third possibility, although of much more speculative nature, is that the elimination of birds with bumblefoot had a similar effect, if there exists an association between size of bird and incidence of this defect.

There is no indication that a plateau has been reached in the size line. It should, however, be noted that, as shown in table 2, the range of individual values in the size line has not been extended upward from that found in the production line. Furthermore, the parent flock of the Kimber birds which were introduced into the University flock averaged, as has already been pointed out, 10.28 cm., a value nearly identical with the mean of the fourth selected generation. Although the four Kimber birds figure as prominently in the pedigrees of the current production flock as they do in the size line, it is probable that up to this point all that selection has accomplished is to reconstitute the original genotype for size of the Kimber flock. In contrast to this, Goodale (*5*, *6*) was able by similar methods of selection to increase the size of mice considerably beyond that of the original range in his foundation stock.

There are two important differences, however, between Goodale's experimental project and the one reported here. In the first place, his size determinations were made on growing animals rather than on adults. It is possible then that some of the increase in size observed by him (but not all, as is obvious from his data) was due to a more rapid earlier growth rate. Difference in growth pattern independent of mature size are known to exist. Thus Lerner and Asmundson (*12*) described two strains of Leghorns equal in adult body weight but differing in weight during the early stages of growth. So far as the present data are concerned, growth-pattern differences are of no consequence, since previously gathered material on this stock (*11*) indicates that growth in length of the shank ceases considerably before the age at which the present measurements were made.

TABLE 2.—*Distribution of individual birds according to shank length by year and line (total population)*

Shank length (centimeters)	Individual birds by year and line								
	1938	1939		1940		1941		1942	
	Production line	Production line	Size line	Production line	Size line	Production line	Size line	Production line	Size line
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
8.1-8.3						1			
8.4-8.6		2				11		2	
8.7-8.9	5	18	1	5		33		16	
9.0-9.2	41	35	3	28	3	91		61	1
9.3-9.5	95	62	15	39	9	106	4	83	5
9.6-9.8	115	71	15	37	25	68	16	56	20
9.9-10.1	63	58	26	18	16	21	27	27	20
10.2-10.4	35	18	15	7	22	13	30	12	28
10.5-10.7	11	7	5	2	7	2	19	1	23
10.8-11.0	3	2	4	1	1		8	1	15
11.1-11.3		1					2	1	7
Total	368	274	84	137	83	346	106	260	119

The second difference between this and Goodale's experiment lies in the considerably greater scale of Goodale's work with many more generations and animals represented (28,000 mice as against less than 500 pullets in the present size line). Whether or not transgression of the original range can be achieved in the size line by further selection remains to be seen. Under Mather's (*15*) interpretation of the effects of selection on polygenically controlled characters, two series of advances in the direction of selection are probable, the first as a result of recombination of whole chromosomes followed by a plateau, and the second trend upward as a result of recombination of genes within chromosomes. In the present material the advance has been continuous and the reserve of genetic variance in size has by no means been exhausted (see below). Hence, it is likely that even the first phase of the progression described by Mather has not yet been completed.

As an indication of the relation of shank length to body weight, it may be stated that the fourth selected generation weighed on an average at the time of measurement 1,903 gm., while the mean weight of the birds in the production line of that year was 1,666 gm.

RESULTS OF CROSSES BETWEEN LINES

Reciprocal crosses between the two lines were made in each of the last 3 years reported upon here. They were designed so that size-line females mated to a given production-line male were full sisters of the size-line male which in turn was mated to the full sisters of the above production-line male. In this manner it was hoped to circumvent to some extent any possible effect of intraline interfamily variability on the results of the crosses. The mean shank length for each of the groups of reciprocal matings is given in table 3. It may be seen that in spite of the design of these matings the results were somewhat erratic, possibly because of the relatively small number of birds involved. The differences between reciprocal crosses in the 3 years are not always in the same direction. The grand mean of the 50 hybrids out of size-line dams was 9.50 cm., while the 115 hybrids out of production-line dams averaged 9.47 cm. This suggests that neither major sex-linked genes nor maternal effect are involved in the size difference between the two lines.

TABLE 3.—*Mean shank length of daughters resulting from reciprocal crosses between the production and size lines*

Year	Sire		Daughters from—			
			Size-line dams		Production-line dams	
	Band No.	Line of origin	Birds measured	Mean shank length	Birds measured	Mean shank length
			Number	*Centimeters*	*Number*	*Centimeters*
1940	R16	Size	28	9.90	28	9.66
	R11	Production	11	9.46	11	9.15
1940	R33	Size	26	9.87	21	9.13
	R25	Production	4	9.53	34	9.63
1941	S31	Size	22	10.02	41	9.67
	S2	Production	10	9.31	27	9.14
1942	T50	Size	38	10.06	25	9.34
	T13	Production	25	9.52	24	9.25

The F_1 values appear to be closer to those for the smaller parent, but whether dominance or other types of nonadditive gene action is involved cannot be determined with the material on hand. The curious fact that the F_1 values in the different years do not seem to reflect the increasing difference between the corresponding parental generations may be laid to the small number of birds involved. Attempts to analyze the data of table 3 by Wright's (*25*) method of estimation of the sire's genotype did not lead to any more successful interpretation, and are not presented here.

ESTIMATES OF GENETIC VARIABILITY

The usual methods of estimating the portion of variance that is due to differences between genotypes of parents are based on the degree of resemblance between relatives. Lush (*13*) has listed several such procedures with particular emphasis on the one employing the intrasire regression of offspring on dam. In the material on hand this method could not be applied without sacrificing a considerable part of the data on the production line since not all dams used in this line fell into the restricted population measured (see above), and hence were of unknown phenotypes. Furthermore, the separate contributions of the sire and the dam to the variance of their offspring cannot be evaluated by this method. Sex-linked genes, should any be involved, would in the case of birds lead to underestimates of the degree of heritability, while maternal effects may on the other hand lead to overestimates. Nevertheless, when this method is applied to the restricted population of the size line, the degree of heritability is found to be equal to about 38 percent. This figure will be referred to again in a subsequent section.

Another of the methods described by Lush, which involves the comparison of the offspring with the average of the population from which their selected parents originated, requires for its efficient use selections in opposite directions and the continuity of ancestors within each line. Neither requirement is met in this material. Furthermore, neither the phenotypes of sires nor those of the population from which they originated are known.

To arrive at an estimate of the hereditary variance in the different populations, it is possible to isolate the genetic variance between families of full sisters without resorting to computation of correlations between relatives. The key to this method lies in the fact that all of the eggs from all of the birds bred each year were incubated in the same machines, the chicks reared in the same brooders, and the pullets housed together irrespective of ancestry. Under this system whatever environmental effects are present would operate entirely at random so far as the ancestry of the birds in any given hatch is concerned. In each year there were four hatches, but the representation of birds of different families in the different hatches was proportional. Hence, there is no reason to believe that members of a family or of a line shared an environment more common than the members of the flock as a whole. Under these circumstances it may be considered that the differences between the offspring of different matings attributable to differences between parents are genetic in nature. To separate further the genetic differences between lines from those within lines, the size line and the production line in each year need to be handled separately. In this manner the total variance of shank length of full-sister families in each generation may be separated into

1. Genetic variance between lines
2. Total variance within each line
    (1) Genetic variance within each line
        (a) Attributable to sires
        (b) Attributable to dams
    (2) Residual variance

The latter (residual variance) includes the nongenetic variance and that part of the genetic variance which is found within families of full sisters.

There are two possible objections to this method. The first of these arises from the fact that each dam was mated to only one sire within a given year. Hence the originally computed variance of offspring between sires includes also the differences due to the contributions of the dams. By calculating the variance of the offspring between dams within sires, the latter may be isolated. By subtracting the figure thus obtained from the original variance between sires the contribution of the sires may be assessed.

The second possible objection may be raised by questioning whether some of the differences between dams are due to nonhereditary maternal effects. Differences in nutritional content of eggs laid by different dams may conceivably affect the growth of birds originating from them. In mammals such maternal influence is obviously more extreme (cf. the previously cited work on swine or the report of Walton and Hammond (*21*) on reciprocal crosses between Shetland ponies and Shire horses). In birds this effect is not likely to be great, since it is known, for instance, that egg size, and hence the limitation of the nutritional supply in the embryonic stage, bears no relation to chick weight beyond 2 to 4 weeks of age (*20*). However, even should there be some such effect, the genetic portion of the variance of shank length within lines between full-sister families could be estimated in the absence of sex-linkage by doubling the portion of the variance attributable to the sire on the assumption that the genetic contributions of the two parents are equal. This follows from the fact that the value for the sire is obtained by subtraction of the variance between dams within sires from the total interfamily variance, and thus is free from any possible maternal influence. As may be seen from the figures presented in table 6, twice the estimated contribution of the sire differs on an average by less than one-half of 1 percent from the estimated total interfamily genetic variance.

ANALYSIS OF VARIANCE OF SHANK LENGTH

Table 4 presents the complete analysis of variance for each year. The significant point in the analysis for the total population of each year is the great increase in the amount of variance due to differences between lines effected by selection. The increase is particularly notable in the last 2 years. As shown in the first line of table 5, in 1942 better than 40 percent of the total variance is attributable to interline differences.

TABLE 4.—*Analysis of variance of shank length (restricted population) for each of the different years*

Source of variance	1938		1939		1940		1941		1942	
	Degrees of freedom	Mean square	Degrees of freedom	Mean square	Degrees of freedom	Mean square	Degrees of freedom	Mean square	Degrees of freedom	Mean square
Between lines			1	4.44	1	3.69	1	34.21	1	45.37
Within lines			322	.188	178	.151	405	.168	340	.177
Total			323	.201	179	.171	406	.251	341	.310
Size line:										
Between sires			1	4.37	2	.370	1	.67	2	1.105
Within sires			82	.144	56	.116	59	.209	83	.222
Between dams			9	.204	6	.033	8	.274	8	.378
Within dams			73	.136	50	.126	51	.199	75	.206
Total			83	.194	58	.124	60	.217	85	.243
Production line:										
Between sires	6	0.145	6	.222	4	.076	9	.186	7	.085
Within sires	361	.190	233	.133	116	.143	336	.113	248	.136
Between dams	35	.289	26	.393	12	.255	37	.340	27	.263
Within dams	326	.180	207	.101	104	.130	299	.085	221	.120
Total	367	.211	239	.185	120	.164	345	.159	255	.155

The average variance within lines remained fairly constant; that within the size line showed a moderate but irregular increase; that within the production line decreased in every year from the variance of the previous year. It is possible that this reduction in variance is due to the elimination of potential breeders from families with extremely long shanks by incorporating them into the size line, although, as may be seen from table 6, the decrease is not in the interfamily portion of the variance.

TABLE 5.—*Distribution of percentage variance of shank length (restricted population) for the years 1939–42*

Source of variance	1939	1940	1941	1942
	Percent	*Percent*	*Percent*	*Percent*
Between lines	6.5	11.7	33.1	42.9
Within lines:				
Between sires	22.4	10.9	19.0	9.7
Between dams	19.1	3.7	20.3	10.1
Remainder	52.0	73.7	27.6	37.3
Total	100.0	100.0	100.0	100.0

The figures for the more specific sources of variance are best examined when placed on a percentage basis. They appear for the total population in table 5, and for each line separately in table 6. There is a considerable amount of variation from year to year in the portions of variance attributable to different sources. This is undoubtedly due to sampling fluctuations, because the number of parents in each generation is small. Certain general trends may, however, be noted. Thus in the total population, as has already been mentioned, the variance between lines shows a great increase. This increase is only in part at the expense of the residual variance.

TABLE 6.—*Distribution of percentage variance of shank length within lines (restricted population) for each of the different years*

Line	Interfamily genetic variance—	1938	1939	1940	1941	1942
		Percent	*Percent*	*Percent*	*Percent*	*Percent*
Size	Attributable to sire	-------	24.3	0	3.5	8.0
	Attributable to dam	-----	5.6	0	4.8	7.2
	Total	...	29.9	0*	8.3	15.2
Production	Attributable to sire	9.4	13.7	11.6	21.7	10.8
	Attributable to dam	5.3	31.7	9.1	24.8	11.8
	Total	14.7	45.4	20.7	46.5	22.6

*Mean squares within dams within sires greater than mean error for total. The reduction in mean squares within sires from that of the total is 6.5 percent.

The amount of interfamily genetic variance within the size line has decreased somewhat from the 29.9 percent observed in the first selected generation. There still is, however, at least half of the original variance present. Because of the sampling fluctuations it is impossible to gage accurately what this means in terms of further progress in increasing shank length.

In the production line the amount of interfamily genetic variance fluctuates greatly. It is, however, likely that the genetic variability in this line has not changed significantly from what it was at the beginning of the experiment.

Comparison of the contributions made by the dams with those made by the sire indicates a rather wide range of fluctuation. This may be due in part to unwitting nonrandom mating within each line, and in part to the small number of parents. On the whole, however, it may be concluded that the contributions of the two parents are nearly equal. This substantiates the finding from reciprocal crosses that neither sex-linkage nor maternal influence are of great importance in the inheritance of size in this material.

It may be recalled that the total genetic variance in the size line as computed by the intrasire daughter-on-dam regression method was 38 percent. The average interfamily heritability in the same population is about 15 percent. The difference between these figures may be considered to represent the average intrafamily genetic variance. Undoubtedly because of the increasing homozygosity of the size line the true value of this constant is higher in the early generations of selection and lower in the later generations. The small numbers preclude the possibility of making such estimates for each year separately. Therefore judgment on the extent of the reduction of the intrafamily part of the variance must be reserved. It is clear, however, that (1) shank length is highly hereditary, and (2) the possibility of further increases in the average shank length by selection in the flock has not been yet exhausted.

SUMMARY

A strain of Leghorns characterized by larger body size, as measured by mature shank length, has been established by selection from a production-bred flock.

The method of selection employed involved progeny and sister testing and led to continued increase in shank length for four generations. The range of individual shank lengths, however, was not extended beyond that of the original flock.

Reciprocal crosses between birds from the line selected for size and birds from the production-bred flock failed to reveal either sex-linked factors or any maternal influence.

Analyses of variance of shank length in the total population for each generation indicated an increase of the portion of variance attributable to the effects of selection, rising to over 40 percent of the total variance in the last generation.

The size line still has a considerable reservoir of interfamily genetic variability, pointing to the possibility of further increases in shank length by family selection.

Estimates of genetic variance of shank length within the Leghorn breed indicate that this character is highly hereditary.

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LENGTH-OF-DAY BEHAVIOR OF NICOTIANA GOSSEI[1]

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INTRODUCTION

On June 21, 1939, seed of a native Australian species of tobacco, *Nicotiana gossei* Domin. (fig. 1), was sent to the writer by Dr. B. T. Dickson, chief of the Division of Plant Industry, Canberra, Australia. The seed was collected by a native connected with the Finke River Mission Station, Hermannsburg, Northern Territory, Australia, about latitude 24° S.

EXPERIMENTAL DATA

To determine the response of *Nicotiana gossei* to length of day plants were first grown in the greenhouse during the winter of 1939, with artificial light to lengthen the short daylight period of winter. In this test, light from one 200-watt, 120-volt, clear Mazda bulb with R L M dome reflector was used from sunset until midnight, affording about 18 hours of unbroken illumination each day. This light suspended about 18 inches above the plants supplied an intensity of 200 to 250 foot-candles.

Seed was sown August 24, 1939. Germination occurred October 5. The tiny plants were pricked off and placed in 4-inch pots, on November 28, then transferred to 6-inch pots January 12, 1940, when the tests began. The results of the tests are shown in table 1.

TABLE 1.—*Responses of Nicotiana gossei to the natural short days of winter in the greenhouse and to supplemental electric light from sunset to extend daily light periods to 18 hours*

[Seed sown August 24, 1939; tests begun Jan. 12, 1940]

Light period and plant No.	Budded	Flowered	Time required to flower	Height
Natural day (10–11 hours):			*Days*	*Inches*
1	February 26	March 7	55	44
2	March 1	March 15	63	34
18 hours:				
1	February 12	February 23	42	42
2	do	do	42	42

Under the conditions of these tests it was evident that with the added light the internodes were shorter and the leaves more numerous and that flowering occurred 2 or 3 weeks earlier than for plants that experienced the natural length of day of wintertime. Since the light conditions of winter in the Washington region are rather unfavorable, tests were continued under the stronger summer sunlight alone.

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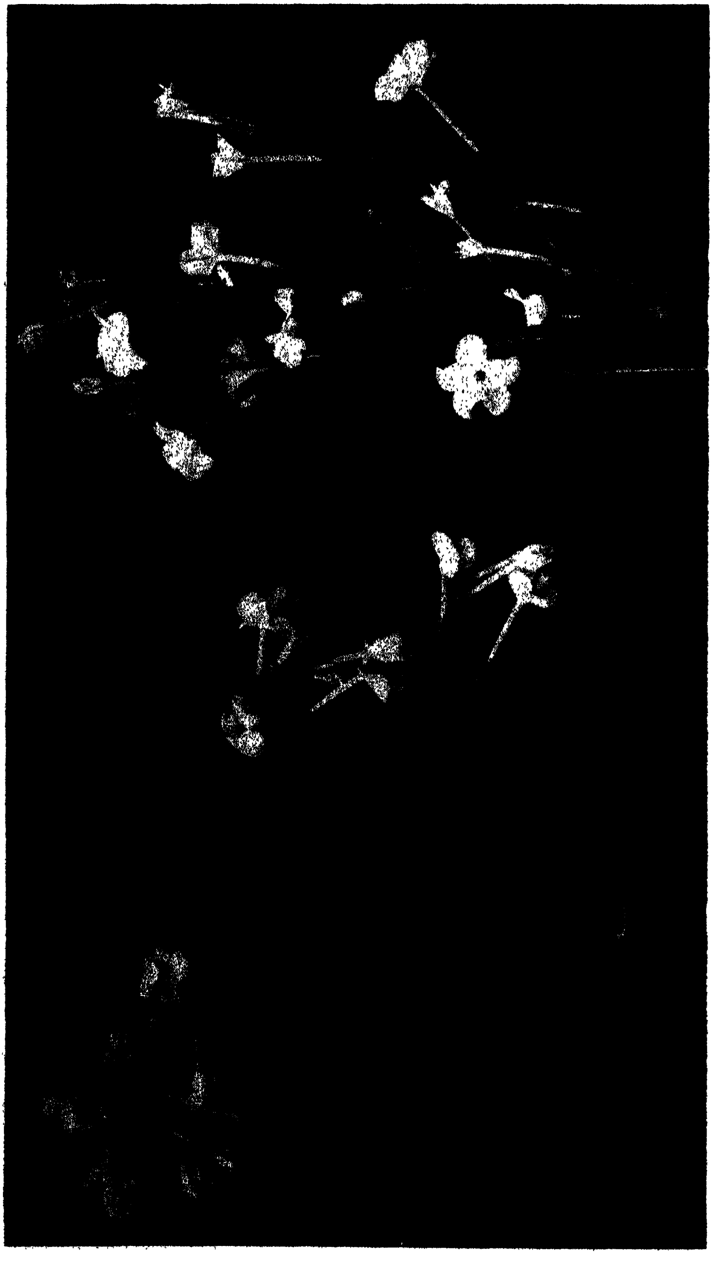

FIGURE 1.—Inflorescence of *Nicotiana gossei*, about one-half natural size. Photographed March 6, 1940.

During the summer of 1940, tests were carried out under natural daylight for all the photoperiods except that of 18 hours. To obtain daylight periods shorter than the full length of day at Washington, D. C., ventilated lightproof dark houses were used. The plants were carried on movable trucks and run into these darkened houses on definite schedules each day to obtain the desired control of daylight exposures.

Since the longest natural day from sunrise to sunset at Washington, D. C., is only 14.9 hours, in order to obtain 18 hours of continuous light each day the plants were exposed, from sunset, to Mazda electric lights from four 200-watt, 220-volt, clear, gas-filled tungsten bulbs with R L M reflectors. These four lights were mounted, one at each corner of a movable square metal frame, so that they were 3 feet apart from center to center of the sides of the frame. This arrangement supplied a light intensity of 300 to 400 foot-candles, as measured by a Weston illumination meter, model 1746, equipped with a Viscor filter to obtain only visible radiation.

TABLE 2.—*Responses of Nicotiana gossei to different daily light periods under natural summer daylight and supplemental electric light*

[Seed sown April 26, 1940; tests begun June 20]

Light period and plant No.	Budded	Flowered	Time required to flower	Height	Leaves to first flower branch
			Days	*Inches*	*Number*
10 hours	July 31	September 20	92	36	32
12 hours	July 12	July 23	33	32	18
13 hours	do	July 29	39	37	26
14 hours	July 1	July 23	33	40	15
18 hours:					
1	July 6	July 17	27	22	12
2	do	do	27	22	14
Full day:					
1	July 17	July 26	40	40	17
2	do	do	40	40	17

Seeds were sown in flats April 26 and germinated May 7. The plants were pricked off into thumb pots June 11 and placed on the tests in 14-quart galvanized buckets June 20, when rosettes were 2 inches high. The results of these tests are shown in table 2.

DISCUSSION AND CONCLUSIONS

From the data of table 1, it will be seen that for the two plants exposed to the short natural days of wintertime an average of 59 days from the beginning of the tests was required for flowering, whereas only 42 days were required for plants receiving 18 hours of light each day.

From table 2 it is seen that the shortened period of 10 hours, where only the summer sunlight was used, delayed flowering until September 20, a period of 92 days from the beginning of the test, June 20. Under the 18-hour photoperiod of this series, flowering took place within 27 days; under the full length of day, flowering took place within 40 days; while the photoperiods of 12, 13, and 14 hours allowed flowering to occur within an average of 35 days (figs. 2 and 3).

The delay in flowering shown by *Nicotiana gossei*, in reality a species of long-day constitution, as shown by the previous tests, would indicate that the species, although now confined to Australia near latitude 24° S., is not necessarily so confined by the day-length factor. Although the maximum length of day there is not more than 13.6 hours, on June 21, this is long enough to induce early flowering, as the tests indicate. The short winter days of its native home, about 10.7 hours on December 21, would be expected to delay flowering.

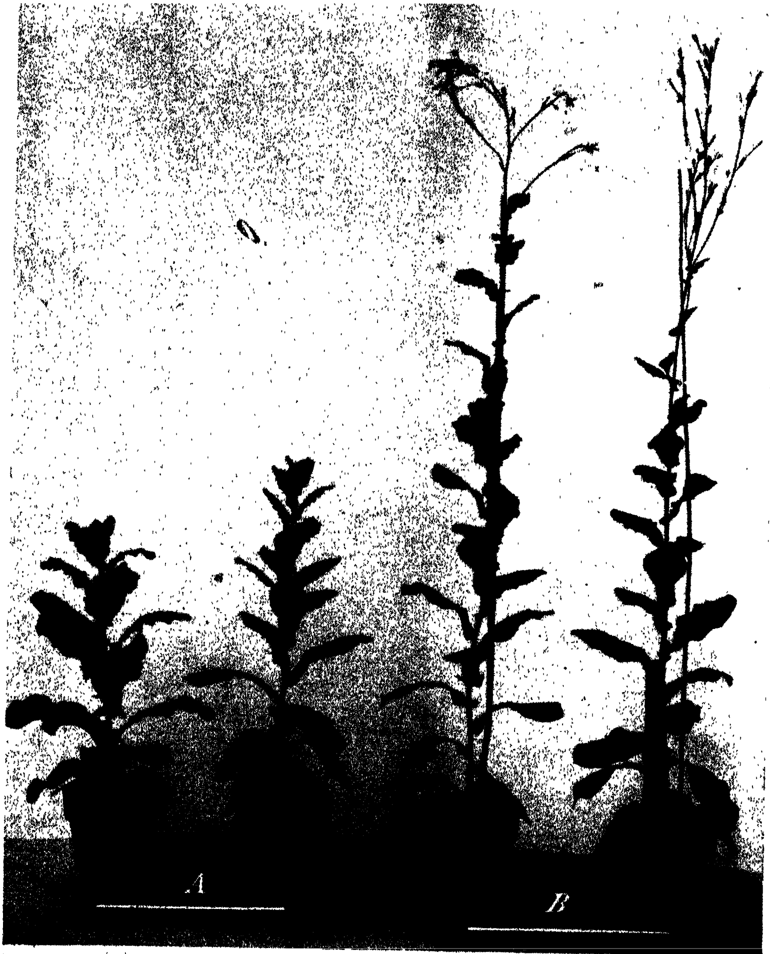

FIGURE 2.—*Nicotiana gossei* grown in the greenhouse at the Arlington Experiment Farm, Arlington, Va., during the winter of 1939–40. Seed was sown August 24, 1939, and tests were begun January 12, 1940. *A*, The two plants that were grown in response to the normal short winter day. These plants flowered March 7 and 15, 55 and 63 days after the tests were begun. *B*, The two plants that were afforded electric light from sunset, giving a constant daily light period of 18 hours. These flowered February 23, 42 days after the tests were begun. Photographed March 6, 1940.

The tendency of *Nicotiana gossei* to hasten flowering in response to long days and to show retarded flowering in response to short days is strongly in contrast with the short-day behavior of the Maryland Mammoth strain of *Nicotiana tabacum.* The genus *Nicotiana* is mainly a group of day-neutral species that flower readily whether the days are long or short. With the exception of the somewhat anomalous Maryland Mammoth strain and a few others showing similar behavior, the writer has found all the commercial varieties of *Nicotiana tabacum* studied to be day-neutral in their flowering requirements. Likewise the species *Nicotiana rustica* may prove to be a day-neutral assemblage.

It is of interest to note that, were it not for ocean barriers, *Nicotiana gossei,* on the basis of its length-of-day requirements, could well have become a continental species with a range into much higher latitudes, provided other climatic and habitat conditions were favorable to its survival.

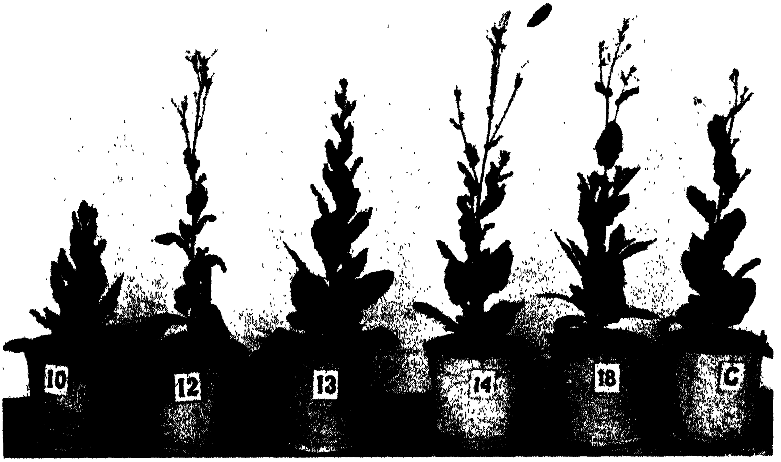

FIGURE 3.—*Nicotiana gossei* grown under different photoperiods during the summer of 1940 at Arlington, Va. Seed was sown April 26, and tests were begun June 20. The plants responded to the various day lengths as follows: 10-hour day, budded July 31, flowered September 20 at 36 inches; 12-hour day, budded July 12, flowered July 23 at 32 inches; 13-hour day, budded July 12, flowered July 29 at 37 inches; 14-hour day, budded July 1, flowered July 23 at 40 inches; 18-hour day, budded July 6, flowered July 17 at 22 inches; full day (*C*), budded July 17, flowered July 26 at 40 inches. Photographed July 25.

Maryland Mammoth tobacco, on the other hand, represents the opposite tendency, and is better adapted to a distribution into lower latitudes if its natural survival depends upon successful seed production before frost. This inherent requirement of the Maryland Mammoth variety for shortened days has called for special methods of handling and, in order to obtain seed to preserve the strain from extinction, various expedients have been resorted to. The rootstocks in some instances have been carried into the greenhouse, where flowering readily takes place in response to the short winter days, or the plants have been grown in movable containers and given artificially shortened days in darkened rooms or cellars. Lastly, the plants have been grown in more southern latitudes of the United States, where they can flower naturally out of doors before destructive frosts occur.

It may not be irrelevant to state here that seeds of *Nicotiana gossei* show a very pronounced rest period after harvest, before germination takes place.

Seed harvested and sown thickly on the same day, September 6, showed only a single germinated plant on October 10, whereas seed harvested in the greenhouse March 19 and sown September 6 showed 13 plants germinated and others appearing on October 10. The seed of March 19, although sown at intervals throughout the summer, gave no germination until the sowing of September 6 was made.

SUMMARY

The flowering of *Nicotiana gossei* Domin., seed of which was obtained from Northern Territory, Australia, is favored by long days and delayed by short days.

Artificial light supplementing the naturally short days of the wintertime at the Arlington Experiment Farm, Arlington, Va., greatly hastened flowering.

Reducing the daily light exposures in summertime by means of darkened houses delayed flowering.

The seeds of *Nicotiana gossei* require a long rest period before vigorous germination can occur.

A majority of the varieties of *Nicotiana tabacum* are day-neutral plants, and the Maryland Mammoth variety and a few other Mammoth strains are the only ones in this assemblage at present known definitely to require short days for flowering.

Nicotiana gossei, on the other hand, so far as known at the present time, is the only species in which flowering is very noticeably hastened by long days.

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THE STRUCTURE AND GROWTH OF VIRGIN BEECH-BIRCH-MAPLE-HEMLOCK FORESTS IN NORTHERN PENNSYLVANIA [1]

By H. ARTHUR MEYER, *assistant professor of forestry*, and DONALD D. STEVENSON *professor of forest research, Pennsylvania Agricultural Experiment Station*

INTRODUCTION

Of the virgin beech-birch-maple-hemlock forests which formerly occupied large sections of northern Pennsylvania and southern New York only remnants now remain. The better known of these in Pennsylvania, namely, Hearts Content, the Tionesta Tract, Cook Forest, and the Ganoga Glen area of the Ricketts estate, have been or are being set aside as parks and scenic areas for public recreational use, and as laboratories for the scientific study of the composition and structure of this forest type. A number of investigators have studied the composition of the Hearts Content, Tionesta, and Cook Forests (*4*, *9*, *13*) [2] as well as the general ecological relationships of this forest type (*5*, *6*). The discovery of several additional virgin stands in Bradford and Sullivan Counties and a knowledge of the virgin forest tracts owned by the Central Pennsylvania Lumber Company near Sheffield, Warren County, led the writers to undertake an investigation of the structure and growth of virgin forests of this type, since data of this sort supply the forester with basic information in the management of uneven-aged stands. Further, it was the desire of the writers to obtain field data of the smaller Bradford and Sullivan County tracts and the Central Pennsylvania Lumber Company holdings before they were logged off. Stand tables of such tracts are irreplaceable and are a valuable source of information for further comparative studies of the virgin forest.

The structure of forest stands has to do both with composition and with diameter distribution. Meyer (*12*), Schnur (*15*), and others investigated diameter distributions in even-aged stands. They employed statistical methods to produce harmonized stand tables for each tree species. Such an application is of value in the construction of yield tables. Diameter distributions in uneven-aged forests have been studied especially by de Liocourt (*8*) and Schaeffer, Gazin, and d'Alverny (*14*) in France. The latter applied the well known graphical J-shaped distribution curve for uneven-aged forest stands to the management of selection forests. More recently diameter distributions of selection forests in Switzerland have been represented by an exponential function. This method was also applied to virgin forests in Mexico (*10*, *11*). Hough (*3*) found that diameter distributions of virgin forests in northwestern Pennsylvania show trends similar in form to managed selection forests in Europe.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 483.

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The application of the results of this study will not be discussed in this paper. It might be pointed out, however, that a knowledge of the balanced structure of an uneven-aged forest is of great importance for practical management. Failure to take into account the desired normal structure of a forest, a structure which should be secured and maintained in order to achieve the goal of sustained-yield management, often leads to a gradual depletion of the growing stock. Data derived from a study of the structure of virgin forests represent a valuable basis for an appraisal of the normal growing stock of uneven-aged forests. Such data should be secured before the last remnants of virgin stands in many sections of the country disappear forever.

EXPERIMENTAL MATERIAL

The location and a general description of the virgin stands included in this study are given in table 1. The stand tables have been compiled and published in the form of a mimeographed research note [3] in order to be available for future reference. In table 2 are listed some of the more important characteristics for each stand which can be numerically evaluated. A total of 419.2 acres of virgin stands were calipered. The distribution of volume by diameter groups (small, medium, and large timber) varies considerably, though each stand shows what is called a balanced distribution of number of trees by diameter classes. It is apparent that the volume distribution by diameter groups follows a certain pattern, but only through a systematic analysis will it be possible to elucidate this variation in the structure of the virgin forests studied.

In computing the cubic foot volume of the various stands one common volume table was applied to all species. A preliminary examination of volume per tree and by species revealed no significant difference between the volumes at different localities, or between the various species of hardwoods. A common volume table for all hardwoods and one volume table for hemlock was then established. It so happened that these two volume tables in turn showed no significant difference; one common volume table was therefore established for all species, as shown in table 3. In spite of the fact that the differences between the various volume tables originally established were statistically not significant, it must be admitted that larger samples of height measurements would probably have shown that some of the observed differences between certain species and localities actuallyare significant. However, for the comparison of the structure of the various virgin stands in terms of cubic feet it is decidedly an advantage to apply one and the same volume table to all species and all stands, since the results of the data from the various stands can then be compared more easily. It should also be said that the yield from cuttings made in these stands would hardly coincide in all cases with the anticipated cubic foot volume per tree, since the actual degree of utilization varies quite widely from one operation to another.

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[3] YOUORSKI, J., and MEYER, H. A. STAND TABLES OF VIRGIN BEECH-BIRCH-MAPLE-HEMLOCK FORESTS. Pa. State Forest School Res. Paper 3. 1942 [Processed.]

TABLE 1.—*General description of virgin stands included in study*

Designation of tract and location	Stand No.	Area	Location and description
		Acres	
McClure: I	1	12.2	In Bradford County; isolated farm woodland.
Hess:			
I	2	14.4	In Sullivan County; farm woodland, rocky site with northern aspect.
II	3	10.8	
Ricketts:			
III	4	9.4	On Wyoming and Luzerne County line in bottom land along Kitchen Creek.
IV	5	5.0	
Rightmeier:			
I and II	6	11.7	In Sullivan County; farm woodland along creek and on gentle slope, deep soil.
III	7	17.6	
IV	8	13.5	
V	9	22.1	
VI	10	9.7	
C. P. L. Larson:			
I	11	34.6	In Warren County; on upland site with western aspect.
II	12	7.4	
C. P. L. Dunham:			
I	13	36.9	In Warren County, on moderate to steep slope with eastern aspect.
II	14	21.5	
III	15	15.3	
IV	16	32.3	
Tionesta:			
II	17	12.5	In scenic area of Tionesta forest along both sides of Cherry Creek, mostly deep and wet soils.
III	18	11.2	
IV-A	19	15.4	
IV-B	20	14.6	
V	21	10.6	
VI	22	14.3	
VII	23	21.4	
VIII	24	9.2	
IX	25	24.2	
X	26	11.4	

TABLE 2.—*Numerical characteristics of individual stands, material 7 inches and over*

Stand No.	Area [1]	Trees per acre	Volume per acre	Distribution of volume by diameter groups (inches)			a [2]	k [2]	b [2]	Proportion of hemlock stems
				7 to 13	13 to 21	21 and over				
	Acres	*Number*	*Cubic feet*	*Percent*	*Percent*	*Percent*				*Percent*
1	12.2	86.1	3,072	28	33	39	0.143	33	31	20
2	14.4	89.2	4,130	18	37	45	.114	24	33	11
3	10.8	78.3	4,171	13	41	46	.072	12	31	6
4	9.4	89.4	3,097	27	45	28.	.175	53	35	14
5	5.0	97.6	5,313	14	31	55	.088	18	33	45
6	11.7	98.4	3,699	25	41	34	.145	41	31	25
7	17.6	113.6	4,226	25	44	31	.145	47	31	25
8	13.5	103.2	3,910	24	40	36	.140	40	31	21
9	22.1	92.0	3,862	20	44	36	.122	28	31	14
10	9.7	63.4	2,156	29	45	26	.180	41	33	21
11	34.6	77.8	4,681	12	29	59	.071	11	33	46
12	7.4	101.4	5,314	15	38	47	.079	17	31	45
13	36.9	66.9	4,331	11	29	60	.073	10	35	54
14	21.5	56.9	3,427	11	35	54	.085	10	35	33
15	15.3	65.7	2,742	21	34	45	.135	23	35	32
16	32.3	74.0	3,593	17	27	56	.116	19	37	51
17	12.5	66.8	3,110	17	41	42	.150	30	41	24
18	11.2	73.3	3,544	17	33	50	.137	27	39	40
19	15.4	94.2	4,508	19	29	53	.131	31	39	33
20	14.6	84.7	5,356	11	24	65	.092	15	39	50
21	10.6	82.1	4,928	13	26	61	.102	18	39	41
22	14.3	75.4	5,040	11	22	67	.083	12	39	38
23	21.4	81.1	4,054	16	34	50	.128	26	39	25
24	9.2	88.5	5,057	16	23	61	.103	19	39	27
25	24.2	86.3	4,293	15	33	52	.134	31	41	26
26	11.4	72.3	4,733	12	33	55	.118	20	41	38

[1] Total, 419.2 acres.

[2] See text for further explanation of these coefficients.

TABLE 3.—*Common local cubic foot volume table*[1]

Diameter at breast height (inches)	Volume[2]	Diameter at breast height (inches)	Volume[2]
	Cubic feet		*Cubic feet*
8	9.315	30	214.3
10	15.83	32	249.9
12	24.38	34	288.4
14	35.14	36	330.3
16	48.23	38	375.5
18	63.78	40	424.1
20	81.90	42	476.1
22	102.7	44	531.6
24	126.2	46	590.8
26	152.6	48	653.5
28	182.0	50	720.0

[1] Logarithmic volume equation $\log V = -1.1733 + 2.3724 \log D$.

[2] Includes stump and bark, but no limb wood.

The finally accepted local cubic foot volume table was plotted on logarithmic paper and a straight line was fitted to the points. The constants a and b of the logarithmic volume equation $\log V = a + b \log D$ are given in table 3.

THE STRUCTURE OF THE VIRGIN FOREST

DISTRIBUTION OF NUMBER OF TREES BY DIAMETER CLASSES

A virgin forest or a virgin stand capable of maintaining its volume must show a balanced distribution of number of trees by diameter classes. The actual growing stock of a forest can be maintained only if the trees which are dying every year are continuously being replaced by trees moving up from the lower diameter classes. It is therefore necessary that the number of trees in successive diameter classes decrease gradually with increasing diameters. In making this statement it is assumed that diameter increment is a slow-changing function of diameter at breast height, which is known to be true. An example of a well-balanced diameter distribution is graphically shown in figure 1. To characterize the structure of an uneven-aged forest means to describe mathematically the form of the distribution of trees as a function of diameter at breast height. It has been found (*10, 11*) that this distribution can adequately be expressed by the distribution function.

$$df = k \cdot e^{-aX} . dX,$$

where k and a are constants, X stands for diameter at breast height, and e for the base of the natural logarithms. Upon taking logarithms it is seen that the logarithm of the number of trees is a linear function of diameter. By plotting the stand table of a forest on semilogarithmic paper in terms of diameter, we therefore obtain a straight line. It may also be stated that the number of trees in successive diameter classes form a geometric progression the ratio of which is found to be e^{ah}, where h is equal to the width of a diameter class. The French forester de Liocourt, who was working with uneven-aged selection forests, was the first to recognize this important fact. For numerical work, it is more convenient to describe a given distribution in terms of the exponential function given above.

The coefficients *a* and *k* have been determined for every stand listed in table 2. A graphical representation of each distribution was made on semilogarithmic paper in order to judge whether each of the calipered areas showed a distribution sufficiently balanced to be used in this study. No areas calipered were rejected, although it might have happened that too small areas had been taken as units, which would have resulted in too erratic a distribution to be used in this kind of work.

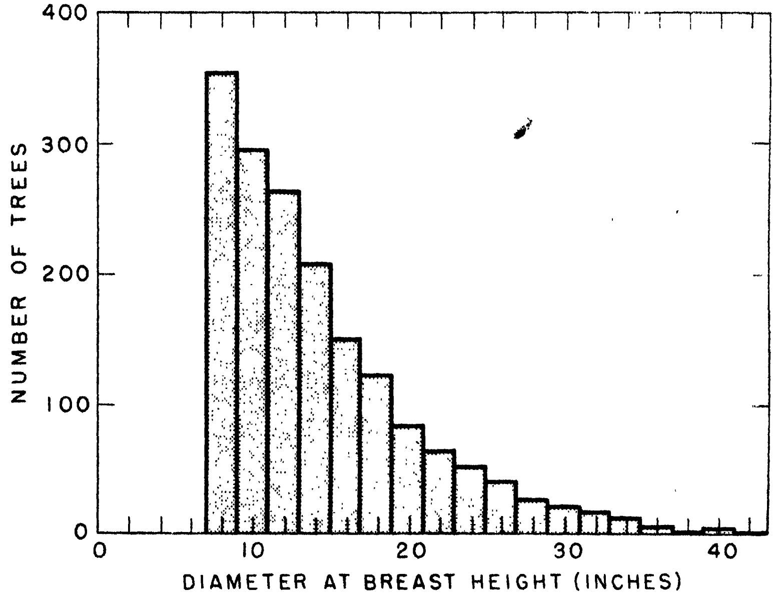

FIGURE 1.—Distribution of trees in stand No. 23, Tionesta scenic area, 21.4 acres.

Before the coefficients *a* and *k* can be calculated it is necessary to determine for each stand the upper diameter limit *b* of the distribution. Regarding the determination of this upper limit it must first be stated that the number of trees in the last highest diameter classes drops off rather abruptly and the points representing the number of trees in these highest diameter classes fall below the straight line that indicates the principal course of the distribution. In order to be able to define an upper limit of the distribution given in terms of the exponential function, the trees of the last few diameter classes are combined in a lower class in such a way that the plotted logarithms of the number of trees in that last class fall within the general trend of the distribution (fig. 2). The upper boundary of this new last class is taken as the upper limit of the distribution. In combining the trees of the last few classes, the actual basal area of these trees is first calculated; it is then determined how many trees of a diameter corresponding to the midpoint of the last class are required to make up the same total basal area, and this number is taken as the frequency of the last class.

Having determined the upper limit b of the distribution, the characteristic coefficients a and k are determined for each stand by the method of least squares. The stand tables are used in their original

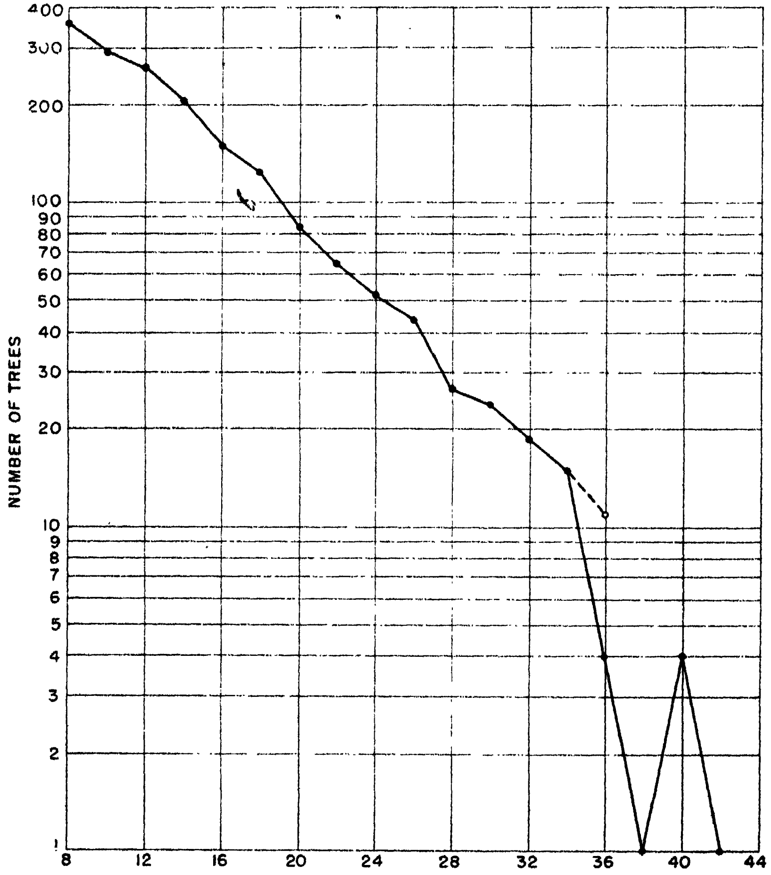

FIGURE 2.—Number of trees represented on semilogarithmic paper, stand No. 23, Tionesta scenic area, 21.4 acres.

form, without first calculating the number of trees per acre. Writing the equation

$$Y = K.e^{-aX}$$

in logarithmic form,

$$\log Y = \log K - a.X.\log e$$

a straight line is fitted to the logarithms of the frequencies Y. The weights of log Y are taken as equal. Details of the numerical computation of a and k are shown in table 4. In the given example the coefficient K refers to an area of 21.4 acres. If the number of trees had first been divided by 21.4 acres it is easy to see that K would be 1/21.4 of the original K. We therefore divide K by the acreage, obtaining a new coefficient which shall be designated by k'. This coefficient refers to an area of 1 acre, in the sense that $k'.e^{-aX}$ gives directly the calculated number of trees in a given 2-inch diameter class. However since we wish to write the frequency distribution in the customary form $df=k.e^{-aX}dX$, so that

$$\int^{X_2} k.e^{-aX}.dX$$

represents the number of trees in a given diameter class with lower and upper limits X_1 and X_2, it is necessary to determine a new and final value k from k', such that

$$k'.e^{-aX_0}=\int_{X_1}^{X_2} k.e^{-aX}.dX$$

In this expression $X_0=\frac{1}{2}(X_1+X_2)$; the formula for computing k is easily found to be as follows, if the width of the diameter classes is equal to 2 inches:

$$k=\frac{a.k'}{e^a-e^{-a}}$$

or, approximately,

$$k=k'/2$$

TABLE 4.—*Computation of coefficients a, k, and b which characterize structure of virgin stand; stand No. 23, Tionesta scenic area, 21.4 acres*

Diameter at breast height (X)	Number of trees (Y)	log Y	X^2	X log Y
8	355	2.550	64	20.400
10	295	2.470	100	24.700
12	262	2.418	144	29.016
14	209	2.320	196	32.480
16	150	2.176	256	34.816
18	124	2.093	324	37.674
20	85	1.929	400	38.580
22	65	1.813	484	39.886
24	52	1.716	576	41.184
26	44	1.643	676	42.718
28	27	1.431	784	40.068
30	24	1.380	900	41.400
32	19	1.279	1,024	40.925
34	15	1.176	1,156	39.984
36	[1] 11	1.041	1,296	37.476
Total	1,737	27.435	8,380	541.310

[1] In the original stand table there were 4, 1, 4, 1 trees in the diameter classes 36, 38, 40, 42 respectively. The total basal area of these trees is equal to 80.70 square feet. The basal area of a 36-inch tree being equal to 7.07 square feet, we find an equivalent of 80.70/7.07=11 trees for the 36-inch class.

Normal equations: $N \log K - a \log e \, \Sigma X = \Sigma \log Y$ ($N=15$)
$\log K \, \Sigma X - a \log e \, \Sigma X^2 = \Sigma X \log Y$

The solution of the normal equation gives $a=0.1280$ $K=1127.1$
therefore $k'=1127.1/21.4=52.67$ and by formulae $k=a\,k'/(e^a-e^{-a})\sim k'/2=26.3$

Upper limit of distribution $b=37$ inches.

ANALYSIS OF VARIATION IN STRUCTURE

When the numerical characteristics of the various virgin stands are compared, it is evident at once that there exists a close relationship between the coefficients, *a* and *k*. For a given value of *a*, which determines the rate at which the number of trees diminishes in successive diameter classes, higher and lower values of *k* indicate a higher or lower relative density between these stands. It is clear, therefore, that when values of *a* are low, which results, in a relatively large amount of heavy timber, the relative density in number of trees per acre must be small. The high correlation between these two coefficients is apparent from figure 3. The coefficient of correlation is equal to 0.9147. Large values of *a* are associated with large values of *k*. This is of special importance for the further evaluation of the data, since it enables us to calculate gradually differing diameter distributions within the range of the possible balanced structures of virgin forests of the beech-birch-maple-hemlock type. Similar correlations between the two coefficients *a* and *k* have previously been found for virgin forests in the higher altitudes of Mexico and for managed selection forests of Switzerland (*10, 11*). It is possible, then, to calculate gradually differing balanced structures of virgin forests by determining the number of trees by diameter classes for distributions characterized by corresponding values of *a* and *k*. From the resulting stand tables it is easy to determine the distribution of basal area, cubic foot volume, or board foot volume. At the same time it is also possible to determine for each pair of the values *a* and *k* the corresponding value of *b*, the upper limit of the distribution. The corresponding value which indicates the proportion of hemlock for the resulting types of distribution may also be determined.

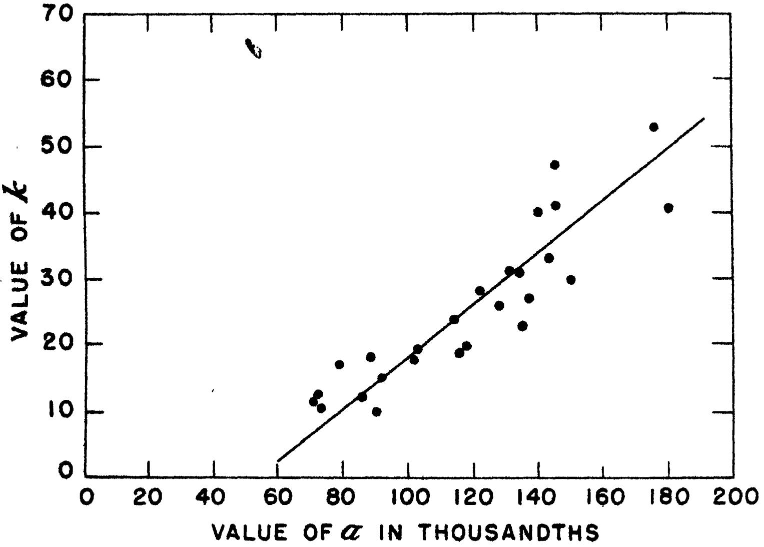

FIGURE 3.—Relation between the characteristics *a* and *k*.

In determining corresponding values of these various characteristics, it is important to realize that no single characteristic may be regarded as a dependent variable. In order to reduce the coefficients to a comparable scale, it is necessary first to express them in deviations from their respective mean and in units of their standard deviations. Considering for the the moment only two coefficients, the values obtained may be plotted in a system of rectangular coordinates and a straight line fitted to the plotted points so that the sum of the squared perpendicular distances of these points from the line becomes a minimum. Working with three coefficients, a system of rectangular coordinates in three dimensional space would have to be used, and the line would have to be fitted to a cluster of points in space, again in such a manner that the sum of the squared residuals from the line would become a minimum. Through analogy, the idea may be carried over into four dimensional space if sets of four coefficients are to be compared, as in the present case. The technique of fitting lines in *n*-dimensional space is well developed and adequately described elsewhere (*1*, *2*, *7*). In the following outline of the various steps to be followed in the computational work only a few comments and explanations will be given. For presentation of the theory of the method the reader is referred especially to the paper of Kendall (*7*).

Designating the characteristics a, k, b, and the proportion of hemlock by X_1, X_2, X_3, and X_4 respectively, the correlation coefficients r_{12}, r_{13}, etc.[4] are first calculated. The numerical values of the correlation coefficients are as follows: $r_{12}=0.9147$; $r_{13}=0.0715$; $r_{14}=-0.5669$; $r_{23}=-0.1581$; $r_{24}=-0.6154$; $r_{34}=0.2694$. While some of the correlation coefficients are not significant, others are significant and highly significant. Since the structure of a stand is mainly determined by the two coefficients a and k, the high correlation existing between these two coefficients should be especially noted. The fact that the correlations between the coefficient b and the other characteristics is low is of minor importance because it merely indicates that the upper limit b of the diameter distribution varies only within narrow limits. The correlations between the proportion of hemlock and a, k, and b respectively are not high, indicating that for a given type of diameter distribution the proportion of hemlock may vary considerably.

The equation of the straight line to be fitted to the observed points lying in a four dimensional space is written in the following form:

$$\frac{x_1}{l_1}=\frac{x_2}{l_2}=\frac{x_3}{l_3}=\frac{x_4}{l_4}$$

where x_1, x_2, x_3, and x_4 represent the four characteristics expressed in deviations from their mean and in units of their respective standard deviations; the symbols l_1, l_2, l_3, l_4 represent the direction cosines of the line. The line passes through the origin of coordinates. It can be shown that the line fitted by least squares as indicated above must actually pass through the point representing the weighted arithmetic averages of the various characteristics. This point may therefore rightfully be taken as the origin of coordinates. As soon as the direction cosines are known it is possible to calculate the coordinates for successive points lying on the line by assigning to either one of the four variables a series of values lying within the range of those actually observed. After expressing these values as deviations from the mean and in units of the standard deviation of the respective characteristic, a set of values of one selected variable, say x_1, is obtained. The corresponding values of x_2, for example, are obtained by multiplying the value of x_1 by the ratio $\frac{l_2}{l_1}$. The resulting values of x_2 must finally be transformed back into the original units. The corresponding values of x_3 and x_4 are found in a similar manner.

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[4] Since the area w of the various stands was different, these coefficients were calculated by the formula $r_{xy}=\Sigma w(X-\bar{X})(Y-\bar{y})/\sqrt{\Sigma w(X-\bar{X})^2 \cdot \Sigma w(Y-\bar{y})^2}$.

As shown by Kendall (*7*), the least square solution for the direction cosines leads to the following equations:

$$\begin{vmatrix} 1-\lambda & r_{12} & r_{13} & r_{14} \\ r_{12} & 1-\lambda & r_{23} & r_{24} \\ r_{13} & r_{23} & 1-\lambda & r_{34} \\ r_{14} & r_{24} & r_{34} & 1-\lambda \end{vmatrix} = 0 \qquad (1)$$

$$\begin{aligned} l_1(1-\lambda)+l_2r_{12}+l_3r_{13}+l_4r_{14}&=0 \\ l_1r_{12}+l_2(1-\lambda)+l_3r_{23}+l_4r_{24}&=0 \\ l_1r_{13}+l_2r_{23}+l_3(1-\lambda)+l_4r_{34}&=0 \\ l_1r_{14}+l_2r_{24}+l_3r_{34}+l_4(1-\lambda)&=0 \end{aligned} \qquad (2)$$

The homogeneous equations (2) can be solved as soon as we know the multiplier λ which is obtained from the characteristic equation (1), representing an equation of the fourth degree in λ. Of the four possible solutions, the largest value of λ must be substituted in equations (2). It is obvious that the direct computation of λ becomes very laborious, if not impossible, as the order of the matrix increases. Through the application of an ingenious method of successive approximations, it is possible, however, to avoid altogether the separate evaluation of (1). The procedure explained by Hotelling (*1*, *2*) is as follows: Multiply the row of the matrix of the correlation coefficients by an initial set of trial values, these being equal to plus or minus 1. Since in the present example some of the correlation coefficients are negative, the trial values for the first, second, third, and fourth row are taken as 1, 1, −1, and −1, respectively. After multiplying successive coefficients in every row by successive trial values (by starting for each row with the first trial value)[5] and adding the results, divide the sums obtained for each row by the largest sum obtained. The resulting quotients are taken as the new trial values and the procedure is repeated by multiplying the new trial values with the original matrix until the quotients become stationary. The first two and the last two steps of the computation are shown below. In actual practice it is, of course, not necessary to write down each individual product when using a calculation machine.

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[5] In other words, the writers would multiply the matrix of the correlation coefficients with the one column matrix $\begin{pmatrix} 1 \\ 1 \\ -1 \\ -1 \end{pmatrix}$.

					First trial value
1	.9147	.0715	−.5669		1
.9147	1	−.1581	−.6154		1
.0715	−.1581	1	.2694		−1
−.5669	−.6154	.2694	1		−1
					Second trial value
1.0000	.9147	−.0715	.5669	2.4101	.8965
.9147	1.0000	.1581	.6154	2.6882	1.0000
.0715	−.1581	−1.0000	−.2694	−1.3560	−.5044
.5669	−.6154	−.2694	−1.0000	−2.4517	−.9120
.	.	.	.	.	.
.	.	.	.	.	.
.	.	.	.	.	.
					Twelfth trial value
.9607	.9147	−.0159	.4814	2.3409	.9608
.8788	1.0000	.0351	.5226	2.4365	1.0000
.0687	−.1581	−.2217	−.2288	−.5399	−.2216
.5446	−.6154	−.0597	−.8492	−2.0689	−.8491
					Stationary quotients
.9608	.9147	−.0158	.4814	2.3411	.9609
.8788	1.0000	.0350	.5225	2.4363	1.0000
.0687	−.1581	−.2216	−.2287	−.5397	−.2215
−.5447	−.6154	−.0597	−.8491	−2.0689	−.8492

In the present case 13 successive iterations were necessary before the quotients became stationary. The convergence can be substantially increased by squaring the matrix of the correlation coefficients and applying the process of successive approximations to the squared matrix. The stationary quotients finally obtained are not yet the desired direction cosines but represent numbers which are proportional to them. Since the sum of the squared direction cosines must be equal to 1, these are found by squaring the quotients 0.9609, 1.0000, −0.2215, −0.8492, taking the square root of the sum of these squares and dividing the result into the stationary quotients. In the present example we obtain

Stationary quotients q	q^2	Direction cosines $=q/1.6412$	
0.9609	0.92332881	0.5855	0.3424
1.0000	1.00000000	0.6093	0.3712
−0.2215	0.04906225	−0.1350	0.0182
−0.8492	0.72114064	−0.5174	0.2677
	2.69353170		
	$\sqrt{2.69353170}=1.6412$		0.9999

As a check, the squares of the calculated direction cosines have been added in the last column of the above computations.

STRUCTURAL TYPES

Since the equation of the straight line fitted to the cluster of points representing corresponding values of the various characteristics of the virgin stands is known, for successive values of one of the characteristics the corresponding values of the other three characteristics can now be calculated. It makes no difference to which one of the four characteristics such arbitrary and gradually differing values are assigned. Geometrically speaking, the coordinates of a series of points lying on the straight line fitted to these points are being determined. Accordingly, some successive values of the characteristic *a* are assigned by taking x_1 equal to −2.0, −1.5, −1.0, −0.5, 0.0, 0.5, 1.0, 1.5, and 2.0. The corresponding values of

$$x_2=\frac{l_2}{l_1}x_1,\ x_3=\frac{l_3}{l_1}x_1,\ x_4=\frac{l_4}{l_1}x_1$$

are given in table 5. In the same table are listed the weighted means of the four characteristics, the standard deviation of each characteristic for an observation of weight one (the weight being equal to the area of a stand), and the standard deviation for an average area of 419.2/26=16 acres. When expressing the values of x_1, x_2, x_3, and x_4 in the original units of the coefficients, it must be kept in mind that the average area of a stand was equal to 16 acres. The values of x_1, x_2, x_3, and x_4 should therefore be multiplied by $s/\sqrt{16}$ and added to the weighted means of the respective coefficients in order to obtain values lying within the range of those actually observed. The various coefficients of *a* corresponding to the assigned values of x_1 are obtained as shown in table 6. By using the mean and the standard deviation of the other coefficients, the corresponding values of all four characteristics which have been used to describe the structure of the virgin forests are obtained in the same way. These values are listed in table 7. It will be noticed that the coefficient *k* corresponding to $x_1=-2.0$ turned out to be negative. Since it is obviously impossible for *k* to be negative, and a deviation from the mean as large as this cannot occur in practice, this coefficient must be discarded. This is easily understood by looking at figure 3, where it is seen that for $a=0.00536$ the line falls below the axis of the abcissa. The remaining seven sets of coefficients characterize seven gradually differing structural types of virgin forests of the beech-birch-maple-hemlock type.

TABLE 5.—*Weighted means of characteristics and standard deviations for observations of weight one, together with corresponding values of x_1, x_2, x_3, x_4*

Characteristic	*a* (x_1)	*k* (x_2)	*b* (x_3)	Proportion of hemlock (x_4)
Weighted mean	0.1134	23.5	34.6	33.2
Standard deviation of observation of weight 1	.1197	45.9	14.0	54.7
Standard deviation of observation of weight 16	.0299	11.5	3.5	13.7
Corresponding values of x_1, x_2, x_3, and x_4	2.0	2.081	−.461	−1.767
	1.5	1.561	−.346	−1.326
	1.0	1.041	−.231	−.884
	.5	.520	−.115	−.442
	0	0	0	0
	−.5	−.520	.115	.442
	−1.0	−1.041	.231	.884
	−1.5	−1.561	.346	1.326
	−2.0	−2.081	.461	1.767

TABLE 6.—*Method of obtaining the various coefficients of "a" corresponding to the assigned values of "x_1"*

x_1	$x_1\cdot 0.1197/\sqrt{16}=0.0299\cdot x_1$	$a=0.1134+0.0299\cdot x_1$
2.0	0.0598	0.1732
1.5	.0448	.1582
1.0	.0299	.1433
.5	.0150	.1284
0	0	.1134
−.5	−.0150	.0984
−1.0	−.0299	.0835
−1.5	−.0448	.0686
−2.0	−.0598	.0536

It is an easy matter to calculate the number of trees per acre and by diameter classes for each structural type. For the diameter class ranging from 7 to 9 inches, the following value is obtained for type A:

$$\int_7^9 47.4e^{-0.1732x}\cdot dx=\frac{47.4}{-0.1732}\left(e^{-0.1732\cdot 9}-e^{-0.1732\cdot 7}\right)$$
$$=-273.67(0.2104-0.2975)=23.8$$

TABLE 7.—*Characteristics for gradually differing structural types of virgin forests*

Structural type	a	k	b	Proportion of hemlock
			Inches	*Percent*
A	0.1732	47.4	34.0	9.0
B	.1582	41.5	34.4	15.0
C	.1433	35.5	34.8	21.1
D	.1284	29.5	35.2	27.1
E	.1134	23.5	35.6	33.2
F	.0984	17.5	36.0	39.3
G	.0835	11.5	36.4	45.3
H	.0686	5.5	36.8	51.4

In table 8 are listed the number of trees per acre and by diameter classes for eight structural types. The total number of trees per acre decreases gradually from type A up to type H, while the proportion of heavy timber becomes successively larger. From table 9 it is seen that a large proportion of heavy timber occurs together with a high percentage of hemlock. This observation is of considerable importance for a clear understanding of the development of these virgin forests. There are two possible ways of interpreting this fact. There may be localities and site conditions on which there is permanently a high proportion of hemlock, while on other sites hemlock is only sparsely represented. The various structural types may also represent various stages in the development of the virgin forest. It is this latter interpretation with which this study is mainly concerned. Field studies have deomonstrated that a gradual change in the composition of the virgin stands takes place. A heavy stand of hemlock with hardwoods in the understory will ultimately break up and be replaced by a stand with only a small percentage of hemlock. In stand 19 of the Tionesta tract, for example, the ground was covered with many decayed hemlock stems all lying in the same direction as a result of a serious windstorm. Part of the existing stand is composed of nearly pure hardwoods. In the shade of hardwoods hemlock reproduction is gradually establishing itself and in decades to come hemlock will probably again become the dominant species in the higher diameter classes. As established from the age of mature hemlock, a period of 200 to 300 years must elapse before a complete cycle in the evolution of a virgin stand is completed. When considering a period of several decades only, however, the structure of a stand as characterized by one of the types A to H remains essentially the same. It may therefore, be said to be balanced, despite the fact that over a period of 200 and more years the composition of the stand undergoes a gradual change. With the exception of the extreme types G and H it is likely that any of the other structural types could be maintained indefinitely if the forest were managed on a selection basis.

TABLE 8.—*Stand tables for gradually differing structural types of virgin forests*

Diameter at breast height (inches)	Trees per acre in stand type -							
	A	B	C	D	E	F	G	H
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
8	23.8	23.5	22.6	21.2	19.0	16.0	11.8	6.4
10	16.9	17.1	17.0	16.4	15.2	13.1	10.0	5.5
12	11.9	12.5	12.8	12.7	12.1	10.8	8.5	4.8
14	8.4	9.1	9.6	9.8	9.6	8.8	7.3	4.2
16	6.0	6.6	7.2	7.6	7.7	7.3	5.9	3.7
18	4.2	4.8	5.4	5.9	6.1	6.0	5.1	3.2
20	3.0	3.5	4.1	4.5	4.9	4.9	4.3	2.8
22	2.1	2.6	3.0	3.5	3.9	4.0	3.7	2.4
24	1.5	1.9	2.3	2.7	3.1	3.3	3.1	2.1
26	1.1	1.4	1.7	2.1	2.5	2.7	2.6	1.9
28	.7	1.0	1.3	1.6	2.0	2.2	2.2	1.6
30	.5	.7	1.0	1.3	1.6	1.8	1.9	1.4
32	.4	.5	.7	1.0	1.2	1.5	1.6	1.2
34	.1	.3	.5	.8	1.0	1.3	1.4	1.1
36				.1	.3	.5	.8	.8
Total	80.6	85.5	89.2	91.2	90.2	84.2	70.2	43.1

TABLE 9.—*Total volume and percentage distribution of volume by diameter groups*

Structural type	Total volume per acre	Distribution of volume by diameter groups			
		Small timber, 7 to 13 inches	Medium timber, 17 to 21 inches	Large timber, 21 inches and over	Proportion of hemlock
	Cubic feet	*Percent*	*Percent*	*Percent*	*Percent*
A	2,810	28	39	33	9.0
B	3,290	24	37	39	15.0
C	3,780	21	36	43	21.1
D	4,330	18	34	48	27.1
E	4,780	15	31	54	33.2
F	5,000	12	29	59	39.3
G	4,740	10	26	64	45.3
H	3,300	8	23	69	51.4

The significance of the various structural types is still better understood when computing the basal area and volume per acre and their distribution over three main diameter groups. In practice it is customary to characterize the structure of a stand by giving the total volume per acre and the percentage of small timber, medium timber, and large timber. The limits of these three main diameter groups are often taken as 7 to 13 inches, 13 to 21 inches, and 21 inches and over. The percentages in basal area and volume are very much the same so that it is sufficient to comment on the results expressed in terms of cubic foot volume, as given in table 9. The corresponding data for basal area are given in table 10. It is interesting to note that the volume per acre increases gradually from type A up to type F, where it reaches a maximum of 5,000 cubic feet per acre. At this point, however, the volume drops rather rapidly to 4,740 and 3,300 cubic feet per acre. Although both of these last types still show a gradual decrease in number of trees by diameter classes, the sudden drop in volume is a clear indication that stands of this structure approach a stage where there is too much heavy timber to allow the presence of a sufficient amount of volume in the smaller diameter classes. The next stage in the development of such a stand is characterized by increased mortality of the overmature hemlock and a subsequent increase of volume in the smaller diameter classes. Since these younger trees will consist mostly of hardwoods the further development will lead to a structure similar to type A.

TABLE 10. - *Total basal area and percentage distribution of basal area by diameter groups*

Structural type	Basal area per acre	Distribution of basal area by diameter groups		
		Small timber, 7 to 13 inches	Medium timber, 17 to 21 inches	Large timber, 21 inches and over
	Square feet	*Percent*	*Percent*	*Percent*
A	80.8	33	39	28
B	92.8	29	38	33
C	105.0	26	37	37
D	117.7	22	35	43
E	127.7	19	33	48
F	131.3	16	31	53
G	121.9	13	28	59
H	83.4	11	26	63

The total volume per acre and its distribution by diameter groups as calculated for the various structural types is a valuable guide in appraising the normal volume of a forest of the beech-birch-maple-hemlock type under sustained-yield management. It is quite evident that a forest manager would not attempt to maintain the largest possible growing stock capable of producing a sustained yield but rather a growing stock similar to that of type A, B, or C. Through periodic cuttings and thinnings it would be possible to modify such a structure, especially as far as composition by species is concerned. The normal growing stock in an uneven-aged forest is not a fixed quantity but a variable which may fluctuate rather widely to suit changing silvicultural and economic conditions. The established structural types represent the best available basis by which to judge the limits within which the balanced volume of an uneven-aged forest may fluctuate without endangering sustained production.

GROWTH

By using increment borings at breast height and ring counts on the stump the average diameter increment for the last 10 years was determined in a number of stands and localities. It would have been too laborious a task to determine the growth separately for every area calipered. The data collected refer to an entire tract. They can therefore not be correlated with the structural type which has been characterized by the various coefficients previously discussed. These coefficients refer to subdivisions of the tracts. It is doubtful, however, if any significant correlations between structure and growth could be established because the growth of an area depends to a considerable extent not only on the structure and composition of the stand but also on site quality. Since on one and the same site the structure may vary considerably (because of the long-term cycle of evolution of the forest) it is likely that differences in growth due to site are overshadowed by differences in structure, and vice versa.

The average diameter increment at breast height, calculated separately for hemlock and hardwoods for a number of tracts, is shown in figure 4. The data obtained from the various areas show relatively small differences. On the whole, no pronounced trend of diameter increment is discernible, the average increment being about the same throughout the diameter classes. The growth of the hardwoods, however, is generally greater than the growth of hemlock. This becomes more evident after balancing the original growth data by fitting a straight line or a parabola to the data shown in figure 4. By using the balanced diameter increment thus obtained, the percentage volume increment by diameter classes, as well as total volume increment and volume increment per acre, are easily calculated. From the 8-inch diameter class up to the 30-inch diameter class the percentage volume increment diminishes from about 2.5 percent to 0.9 percent for hemlock and from about 3.0 percent to 0.7 percent for hardwoods. In the smaller diameter classes the relative growth of hemlock is therefore smaller than that of the hardwoods, while the reverse is true for the higher diameter classes.

Additional data on volume increment in percent of volume, and volume increment per acre, are given in table 11. For the total volume of the trees 7 inches in diameter and over, the percentage volume increment of hemlock is consistently lower than that of the hardwoods. The total volume increment per acre ranges between 44 and 67 cubic feet, the average of all tracts combined (weighted according to area calipered) being equal to 50.7 cubic feet. This increment represents gross increment which, over a period of years, will be largely offset by the annual mortality. It is quite evident that the same forest would yield a decidedly larger increment per acre with systematic management under a group selection or group shelterwood system, while at the same time the normal growing stock per acre would probably be somewhat smaller than the actual growing stock in the virgin stands. However, even with the same amount of total volume per acre, the distribution of the volume by diameter classes would be different, more volume being in the lower diameter classes and less in the upper diameter classes.

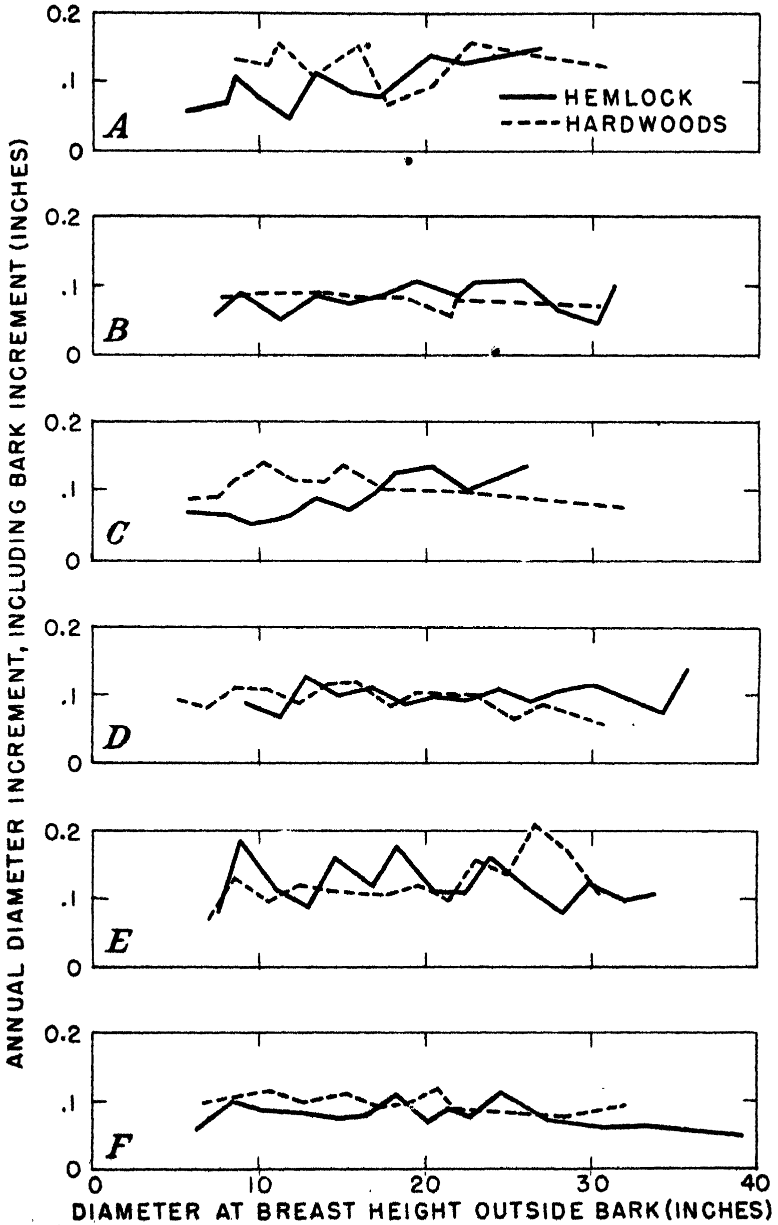

FIGURE 4.—Annual diameter increment, including bark increment, in terms of diameter at breast height outside the bark for trees on various tracts: *A*, Hess; *B*, Ricketts; *C*, Rightmeier; *D*, Larson; *E*, Dunham; *D*, Tionesta.

SUMMARY

By the use of the distribution function $df = k \cdot e^{-aX} \cdot dX$ the diameter distributions of various virgin stands of the beech-birch-maple-hemlock type have been compared statistically. A high correlation between the two coefficients *a* and *k* was found to exist. Large values of *a* are associated with large values of *k*. This important relationship enabled the calculation of gradually differing diameter distributions within the range of the possible balanced structures of virgin forests. For a given value of *a*, which determines the rate at which the number of trees diminishes in successive diameter classes, higher and lower values of *k* indicate a higher and lower density between these stands. In addition to the coefficients *a* and *k*, the stands were further characterized numerically by the upper diameter limit *b*, and the proportion of hemlock. These characteristics proved in turn to be correlated between themselves as well as with *a* and *k*.

By applying statistical methods, seven sets of four coefficients (*a*, *k*, *b*, and proportion of hemlock) were obtained to characterize seven gradually differing structural types of these virgin forests. The total number of trees per acre was found first to increase and then to decrease gradually from type A up to type H, while at the same time the proportion of heavy timber became successively larger. A large proportion of heavy timber occurred together with a high percentage of hemlock. This conforms with the view that the various structural types represent to a large extent different stages in the long time development of the virgin forests. A heavy stand of hemlock with hardwoods in the understory will ultimately break up and be replaced by a stand of hardwoods with only a small percentage of hemlock. However, because of the relatively low correlation between the proportion of hemlock and the characteristics *a*, *k*, and *b*, the proportion of hemlock may deviate considerably from the average values given for the different structural types.

TABLE 11.—*Volume increment per acre and volume increment in percent of volume for hemlock and hardwoods*

Tract	Volume increment per acre			Percentage volume increment		
	Hemlock	Hardwoods	Total	Hemlock	Hardwoods	Total
	Cubic feet	*Cubic feet*	*Cubic feet*	*Percent*	*Percent*	*Percent*
Hess	5.3	61.4	66.7	1.4	1.6	1.6
Ricketts	15.1	28.8	43.9	1.0	1.3	1.1
Rightmeier	14.2	42.4	56.6	1.3	1.6	1.5
Larson	32.9	23.1	56.0	1.1	1.4	1.2
Dunham	31.5	22.4	53.9	1.3	1.7	1.5
Tionesta	18.6	27.3	45.9	.7	1.5	1.0

It was found that the volume per acre increases from type A up to type F, where it reaches a maximum of 5,000 cubic feet per acre. The volume then drops to 4,740 and 3,300 cubic feet per acre, a further indication that these types apparently represent a somewhat over-aged condition of the virgin forest. Stands of this structure have relatively too much heavy timber to allow for sufficient volume in the lower diameter classes. The next stage in the development will be characterized by an increased mortality in the larger diameters and a subsequent increase of volume in the smaller diameter classes. The tables 9 and 10, which show the percentage distribution of volume and basal area by three main diameter groups, explain this situation more clearly.

The structural types represent a basis by which to judge the limits within which the balanced volume of an uneven-aged forest of the birch-beech-maple-hemlock type may fluctuate while the structure of the forest remains sufficiently balanced to insure sustained production. It may be pointed out, however, that the normal structure of a managed forest of the type here considered may be somewhat different from that of the virgin forest, having probably a relatively higher volume in the lower diameter classes.

Analysis of growth showed that the percentage volume increment diminishes with increasing diameter from about 2.5 percent to 0.9 percent for hemlock and from about 3.0 percent to 0.7 percent for hardwoods. The gross volume increment per acre ranges between 43.9 and 66.7 cubic feet, the average of all stands calipered being equal to 50.7 cubic feet. This gross increment is, in part, offset by the current average mortality. As a stand gradually changes from one structural type with a low volume per acre to another with a relatively high volume per acre, the gross increment will exceed the current mortality. In the later stages of this development, when the volume again decreases, the current mortality will be greater than the current gross increment.

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WEARING TESTS ON FABRIC BLENDS OF NEW AND RECLAIMED WOOL FIBER[1]

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INTRODUCTION

Since the curtailment of the use of new wool for civilian needs was effected by the Office of Production Management (January 1942) and later by the War Production Board, the question of the relative serviceability of fabrics containing various blends of new and reclaimed wool is becoming increasingly important. In addition, legislation[3] has given the ultimate consumer access to information, in the form of labels, as to the percentage and type of wool in a specific article; however, such information is of little practical value unless a reasonably correct interpretation of the factual material is possible. In 1940, service of performance tests of garments made of dyed flannel fabrics of various blends of new and reclaimed wool fiber were begun at the South Dakota Agricultural Experiment Station. Certain chemical and physical properties of the fiber and fabric before dyeing had been determined at an earlier date and subsequently reported by Bailey (*4*).[4]

Certain questions arise: Is it possible to predict the wearing qualities of a fabric from laboratory tests or do individual differences in wear habits tend to mask differences in fabric? What chemical changes in the fiber of wool accompany storage and wear of fabrics? How do garments wear out? It is not to be expected that these questions will be answered by this study alone, but need for further investigation of possible trends should be indicated.

As a review of the literature shows, actual service tests are coming to the fore, not to take the place of, but to supplement, and if possible to prove the reliability of predication of service by laboratory tests (*7, 8, 13, 15*). Sommaripa (*15*) has concluded that serviceability tests are sensitive enough to show differences in performance of given fabrics. Although extensive studies have been made of wool fiber and subsequent fabric characteristics (*4, 10, 11, 12*), very little has been published on actual wearing tests of wool fabrics of known fiber history. The Bureau of Animal Industry of the United States Department of Agriculture in cooperation with the Bureau of Home Economics, in 1937, reported a study of serviceability tests on blankets made from four blends of wool (*7*). There are in progress at Government laboratories studies (*5*) involving a comparison of the serviceability of blankets prepared from blends of three-eighth blood new wool with varying amounts of good- and poor-quality reworked wool,

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[3] The Wool Products Labeling Act, effective July 15, 1941.

[4] Italic numbers in parentheses refer to Literature Cited, p. 500.

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a comparison of the serviceability of blankets containing various percentages of reworked wool and mohair with new wool, and the serviceability of suitings made from new wool and blends of new wool with reworked wool and spun rayon. All reports emphasize the need of knowing the fiber history and details of fabric manufacture.

MATERIALS

The flannel fabrics used in this investigation were woven at the Lowell Textile Institute[5] and have a 2 by 2 even twill construction. Uniform blends were used for both warp and filling yarns. Further details of fiber properties and manufacture of these fabrics have been reported by Bailey (*3*). In brief, new wool from Rambouillet sheep raised at the South Dakota State College and high-quality pastel sweater clippings purchased on the market by the Lowell Textile Institute were blended in various proportions. Fabric No. 100 contains 100 percent new wool, fabric No. 75 is made of approximately 75 percent new wool and 25 percent clippings, fabric No. 50 is made of approximately 50 percent new wool and 50 percent clippings, fabric No. 25 is made of approximately 25 percent new wool and 75 percent clippings.

According to labeling definitions of the Wool Products Labeling Act, "reprocessed wool" is the fiber that results "when wool has been woven or felted into a wool product which without ever having been utilized in any way by the utlimate consumer, subsequently has been made into a fibrous state." The sweater clippings may not be classified as "reprocessed wool" but simply as "wool," since under "wool" are included "yarns and other wastes broken off by or entangled in the combing, drawing and spinning processes, and unused knit wool products made of new wool, such as sweater clippings. These partially processed wastes are included under the term 'wool' on the basis that the damage and deterioration which they undergo in being reduced to a fibrous state for reuse, is not sufficient to seriously diminish their original, natural intrinsic protective and service qualities" (*1, p. 19*). It may be concluded that differences found between blends of new wool and the reclaimed sweater clips used in this study would tend to be exaggerated if "reprocessed" wool as defined by the Wool Products Labeling Act and of the same original quality as the clips were used. The term "reclaimed wool" (not used in labeling rules) will be used to indicate the sweater clippings in this study since it is misleading to call it simply "wool."

Following the fiber and fabric studies previously mentioned, portions of the four fabrics were dyed navy blue by a commercial dyer who used identical dyestuffs and processes for each fabric. The description given of the dyeing process[6] is as follows: Wetting out of the material was executed in weak ammonia water for 15 minutes at 110°–120° F. and then in two fresh water rinses at the same temperature. The material to be dyed was then immersed in a water bath (140° F.) to which was added 10 percent Glauber salt and 3 percent acid alizarin blue. The temperature of the water was brought to 195° F. in ½ hour and held there for an additional 1¼ hours. The dyed material was rinsed until the water was clear.

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[5] Lowell Textile Institute, Lowell, Mass.

[6] Dyer's specifications.

Fabric yardage losses incurred in dyeing range from approximately 3.1 to 5.8 percent. The largest percentage loss was found in fabrics No. 100 and No. 50; the smallest in fabric No. 75. Accompanying these losses in fabric yardage there appeared a significant (1 percent level) decrease in warpwise breaking strength (strip test) for all fabrics. Fillingwise breaking-strength values were inconsistent in their variation. Only fabric No. 75 showed a decrease in bursting-strength value. The usual evidences of shrinking were present in that thickness and number of yarns per inch increased for both fabrics.

METHODS

WEARING TESTS

Twelve four-gore skirts were made from the four navy blue wool flannel fabrics and issued to women students at South Dakota State College who agreed to cooperate in the wearing tests. A relatively simple-to-make, conservative, and well-fitting skirt design was chosen to facilitate subsequent fabric sampling and to avoid as far as possible, localized wear due to construction or design details. The selection of college girls made rather close supervision of the wearing periods possible, yet allowed full play of individual wearing habits under ordinary wearing conditions. It was decided that all the garments should be withdrawn from service for testing at the end of the time interval in which one garment showed definite evidences of wear. Accordingly, each skirt was worn a total of 1,000 hours, which approximates the length of time equivalent to the wear a college girl would ordinarily give a skirt during a school year. Careful records of hours worn, type of activity, brushing, pressing, evidences of wear, and damage to garment were kept by each individual wearing one of the skirts. After each 150-hour period the skirts were inspected. Measurements were taken both before and after dry cleaning to indicate stretching or shrinking. Then shirts were dry-cleaned commercially, always at the same establishment where standard procedures were assured.

In the dry-cleaning treatment [7] a solvent (Stoddard solvent, U. S. Department of Commerce commercial standard CS3–40), was circulated through garments placed in a cylindrical washer. A filter aid was added to the solvent after which the solvent was drawn continuously through a filtering device until clear. A stock solution consisting of 2 parts soap, 1 part water, and 1 part solvent was run on the batch for approximately 20 to 30 minutes. Nearing the end of the soap run, more filter aid was added and the solution was again drawn through a filter until clear. The garments were dried and deodorized in a tumbler and pressed with an ordinary steam press.

PHYSICAL MEASUREMENTS OF FABRIC AND GARMENTS

Breaking strength (strip test) and elongation, bursting strength and elongation, fabric thickness, number of yarns per inch, yarn number, and yarn twist measurements were made on the fabrics as received from the dyers and on control fabrics which were stored for a time equivalent to the skirt-wearing period. All physical measurements of yarn and fabric were made in a laboratory maintaining a temperature of 70° F.±2° and a relative humidity of 65±2 percent. Standard procedures of the American Society for Testing Materials (*2*) were used throughout, unless otherwise noted. The testing equipment included a motor-driven Scott tester for breaking-strength and elongation determinations; a ball-burst attachment for the Scott tester, using 1-inch steel balls for burst-strength and elongation determinations; a Schiefer compressometer, the presser foot being 0.375 inch in diameter and the pressure equal to 3.4 pounds per square inch, for thickness measurements; a micrometer yarn counter for number of yarns per inch; a Suter yarn numbering balance (results used in the adjustment of tension for yarn-twist measurement); and a hand-turned new-type Suter precision twist tester for the determination of twists per inch.

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[7] Dry-cleaning specifications.

Similar measurements, with the exception of yarn twist and yarn number, were made on material from the 12 skirts. It was necessary to curtail the number of physical tests used to measure the relative wearing qualities of the 4 fabrics because of visible evidence of localization of wear.

In order to determine fabric strength in various parts of the skirt, each skirt was divided into three sections, designated as top, middle, and bottom. The top section included the skirt area extending from the waistline to the approximate top of the thighs, or to the bend of the skirt if one were in a sitting position. The middle section extended down approximately two-thirds the length of the skirt, and the bottom section included the lower part of the skirt. Care was taken to approximate the same relative sampling location regardless of the size of the skirt. Five breaking-strength samples, 1¼ by 6 or 7 inches, warpwise and fillingwise, were cut from both the front and back of the skirt for each of the three sections. For calculation, it was assumed that the wear on the back left gore, top section, was similar to that on the back right gore, top section. It was suggested that wet breaking-strength measurements might show greater differences than dry breaking-strength measurements; however it was impossible to include both. In addition, it is well known that wool loses strength when wet and under normal conditions wool garments are worn in a more or less dry state.

Ten bursting-strength samples, 4 by 4 inches, were cut from each skirt, the sampling being scattered to represent, as far as possible, all parts of the skirt and to avoid inclusion of indentical warp and filling yarns and identical placement with reference to skirt seams. By such a method the composite bursting strength of the worn fabric should be indicated, or if inadequate sampling were suggested by statistics, real bursting-strength variation in different parts of the skirts would be intimated.

Fabric-thickness and yarn-count (number of yarns per inch) determinations were made on each of the bursting-strength samples.

CHEMICAL ANALYSES

Moisture, sulfur, nitrogen, and ash content of the four blends were determined after (1) fabrics were received from the dyers, (2) after storage, and (3) after the total wear period. Samples of the wool fiber used in the analyses were obtained in a random manner for all blends. A drying oven maintained at 105° C. was used to bring 0.5- to 1.0-gm.

samples for moisture determinations to constant weight. These samples were transferred to a Parr bomb for total sulfur measurement. Total nigrogen present was determined by the Kjeldahl method. Samples were ignited and ashed to constant weight, from which the percent of ash was determined. All percentage calculations of sulfur, nitrogen, and ash were based on moisture-free weights.

PRESENTATION AND INTERPRETATION OF DATA

COMPARISON OF BLENDS

By appearance alone it would be difficult, if not impossible, to distinguish between the four blends, but physical tests leave little doubt as to their dissimilarity. Throughout these blend comparisons it is interesting to keep in mind that it was reported (*3*) that the average length of the new fiber was 40 percent greater than that of the reclaimed wool and the average number of crimps per inch was more than 10 percent greater in the new wool. No difference in average contour ratio was found in the wool. The physical-test data for the dyed flannel fabrics appear in table 1.

Mean strip breaking-strength values, which range from 32.4 pounds for blend No. 100 to 17.4 pounds for blend No. 25 warpwise, and 27.7 to 16.7 pounds, fillingwise, indicate real strength differences according to a *t* test among all four blends, increasing progressively from blend No. 100 to No. 25. Based on the breaking-strength values for the 100-percent new-wool fabric, there were successive losses in warp strength which amounted to 12, 28, and 46 percent and which resulted from the increased percentage of the reclaimed wool per fabric. In filling strength, the losses range from 18 to 32 to 40 percent, indicating a more decisive initial loss due to blending but a tendency to level off more rapidly than did warp strength.

Bursting-strength determinations also reveal a significant strength decrease from No. 100 to No. 25. Figure 1 graphically illustrates the percentage strength of the four blends as evidenced by breaking- and bursting-strength tests.

Fabric elongation, as shown by strip-elongation determinations, decreases somewhat with increasing percentage of reclaimed wool. Because of the greater number of shorter fibers in the fabrics containing the reclaimed wool, this was to be expected. Warpwise strip elongation gave evidence that fabrics No. 100 and No. 75 had a greater fabric elongation than No. 50 and No. 25. In the filling direction, fabric elongation decreased significantly from fabric No. 100 to No. 50. Comparison of warpwise elongation and fillingwise elongation for each blend shows a real difference only for blends No. 100 and No. 75. Bursting-strength elongation for all fabrics was the same, except for No. 25 which was somewhat less.

The number of yarns per inch for all fabrics decreased only slightly throughout the series, both warpwise and fillingwise. Fabric thickness was approximately the same with the exception of blend No. 25, which was significantly (1-percent level) thinner than the other fabrics. Yarn twist and yarn number (typp system) for the four fabrics was very similar, except that yarn twist for fabric No. 25 was somewhat greater than that of other blends.

TABLE 1.—*Physical-test data for dyed flannels, 1941*

Test	Unit of measure	No. 100				No. 75				No. 50				No. 25			
		Warp		Filling		Warp		Filling		Warp		Filling		Warp		Filling	
		Mean	Standard deviation	Mean	Standrad deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
Strip breaking strength	Pounds	32.4	0.97	27.7	0.68	28.6	0.84	22.8	1.32	23.4	0.91	18.7	0.48	17.4	0.52	16.7	0.82
Bursting strength	Pounds	81.10	1.89			69.70	3.44			61.40	4.14			53.35	1.96		
Strip breaking-strength elongation.	Inches	.92	.06	1.06	.08	.84	.05	.96	.05	.72	.04	.75	.05	.66	.05	.69	.09
Bursting-strength elongation.	Inch	.38	.13			.38	.13			.38	.13	[illegible]		.28	.08		
Yarns per inch	Number	45.5		40.0		45.3		38.6		43.4		40.2		43.9		39.9	
Fabric thickness	Thousandths of an inch.	35.45	.98			34.95	.37			34.40	.91			32.25	.26		
Yarn twist (per inch)	Number	13.20	1.04	12.69	.51	12.82	.72	13.02	.92	12.48	.92	12.36	.58	12.57	.88	12.86	1.12
Yarn No.	Typp	6.35		6.25		6.90		6.75		6.78		6.88		7.94		7.56	

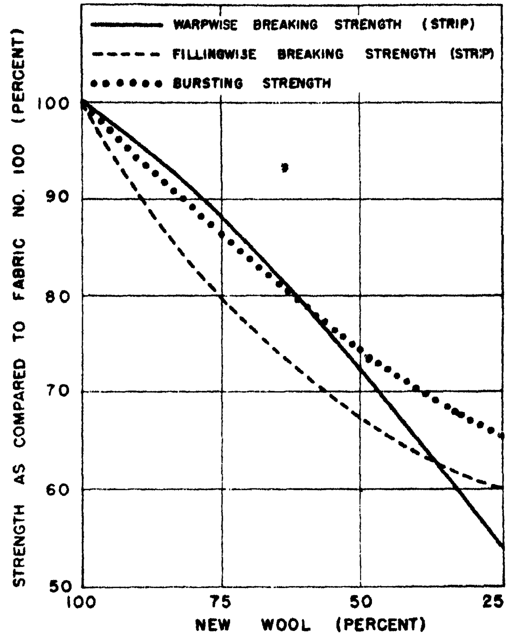

Figure 1.—Warpwise and fillingwise breaking-strength and bursting-strength values, according to percentage composition, of the four fabric blends after dyeing.

Chemical analyses of the four fabrics after dyeing reveal, in general, several trends as seen in table 2. The percentage of ash for fabrics No. 100 and No. 75 is somewhat less than that for fabrics No. 50 and No. 25. Both nitrogen and sulfur content tend to be lower for the fabrics containing large percentages of the reclaimed wool. Others (*3*, *7*) have observed the same general trends.

Table 2.—*Percent total ash, nitrogen, and sulfur calculated on a miosture-free basis for the 4 fabric blends after dyeing, storage, and wear*

Treatment and fabric No	Ash	Nitrogen	Sulfur
	Percent	*Percent*	*Percent*
Dyed:			
100	0.77	16.64	3.71
75	.81	16.65	3.64
50	.87	16.50	3.65
25	.85	16.31	3.62
Stored:			
100	.87	16.00	3.55
75	.98	15.83	3.57
50	.76	15.87	3.52
25	.97	16.00	3.52
Worn:			
100	.94	15.62	3.50
75	.88	15.76	3.57
50	.91	15.68	3.53
25	1.04	15.55	3.51

TABLE 3.—*Physical-test data for stored, dyed flannels, 1942*

Test	Unit of measure	No. 100				No. 75				No. 50				No. 25			
		Warp		Filling		Warp		Filling		Warp		Filling		Warp		Filling	
		Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
Strip breaking strength	Pounds	33.7	1.08	28.1	1.29	29.8	1.25	23.8	1.58	24.0	0.72	19.8	0.75	19.2	0.42	17.6	0.77
Bursting strength	Pounds	75.8	1.89			66.2	3.23			58.8	2.41			49.8	2.14		
Strip breaking-strength elongation	Inch	.81	.03	.87	.07	.70	.05	.79	.07	.63	.05	.64	.05	.50	.05	.56	.05
Bursting-strength elongation.	Inch	.28	.08			.28	.08			.25				.40	.13		
Yarns per inch	Number	44.5		39.5		43.4		39.0		43.0		38.7		43.0		38.4	
Fabric thickness	Thousandths of an inch.	37.4	1.03			38.2	.48			37.2	1.06			35.4	.84		
Yarn twist (per inch)	Number	12.54	.46	11.98	.80	11.98	.86	11.60	.67	11.36	.76	11.34	.82	11.68	.86	11.48	.84
Yarn No.	Typp	6.34		6.10		6.75		6.46		6.60		6.76		7.83		7.35	

EFFECT OF STORAGE

Control fabrics, stored for a time equivalent to the skirt wearing period, were kept at room temperature and in thoroughly sealed boxes to prevent moth infestation. Several differences in physical-test values between the characteristics of the dyed fabrics before and after storing are noted. Physical-test data for the stored fabrics are given in table 3.

Comparisons of breaking strength indicate a slight increase after storage for all warpwise and fillingwise measurements, although this difference is statistically significant in only two instances, No. 25, warpwise (1-percent level) and No. 50, fillingwise (5-percent level). On the other hand, bursting-strength measurements of the two groups reveal a consistent decrease in mean strength for all stored fabrics, differences which are significant only for fabrics No. 100 and No. 25. Fabric thickness increased upon storage, whereas number of yarns per inch, yarn twist, yarn number, strip elongation, and bursting-strength elongation varied within the range of sampling error.

EFFECT OF STORAGE AND WEAR ON CHEMICAL COMPOSITION OF THE BLENDS

Total ash content for fabrics No. 100 and No. 25 appears to increase after storage (approximately 1 year) and even more so after wear. Fabrics No. 75 and No. 50 show an increase in ash content after the wearing period, but storage effects are inconsistent. A decrease in nitrogen and sulfur is observed following both the storage and the wearing periods. There is some indication that fabric No. 25 is consistently low in both nitrogen and sulfur. Based upon these values, evidences of chemical deterioration are, in general, more pronounced after the wearing period than after the storage period.

EFFECT OF WEAR ON GARMENTS

To interpret the breaking-strength values for both warp and filling directions of the worn materials, the analysis of variance (*6*, *9*) was used. Previous to its calculation, homogeneity of error was determined by Bartlett's method (*14*). Variability of the fillingwise breaking strength within skirt sections was greater than could be explained by errors of sampling. Although heterogeneous, these variable data are the only values for the description of fillingwise strength and since the main effects are of more concern than the interactions, in this analysis of variance these fillingwise-strength data were used. However, the heterogeneous nature of these values must be kept in mind during the interpretation that follows. In all other instances homogeneity of error was found. While this statistical treatment may be considered elaborate because of the limited number of replicates involved, it is hoped that it may prove a guide in future studies of this kind.

The means and standard deviations for warpwise and fillingwise breaking-strength determinations, in pounds, for the three sections in each of three skirts and for the four fabric blends are given in table 4. The analyses of variance data used in the interpretation of the warpwise and fillingwise breaking strengths are given in table 5.

Table 4.—*Means and standard deviations of warpwise and fillingwise breaking-strength determinations for 3 sections in each of 3 skirts made of blended fiber, 1942*

Fabric No. and skirt section	Warp						Filling					
	Skirt No. 1		Skirt No. 2		Skirt No. 3		Skirt No. 1		Skirt No. 2		Skirt No. 3	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
	Lbs.	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*
No. 100:												
Top	33.70	1.16	33.30	1.03	34.80	0.82	29.20	1.78	28.95	1.24	29.35	0.82
Middle	35.15	.41	34.30	.92	35.50	.71	30.25	1.75	30.50	.67	30.10	.88
Bottom	34.30	.79	34.00	.47	35.15	.67	31.80	2.16	30.70	2.00	31.05	1.21
No. 75:												
Top	29.05	.60	28.30	1.08	28.55	1.77	24.05	1.38	25.80	1.03	24.50	1.20
Middle	29.35	.94	28.40	1.35	29.60	.81	25.00	.88	25.05	.76	25.80	.59
Bottom	29.20	.75	28.50	.88	29.30	.89	25.00	.85	25.90	.78	26.05	.80
No. 50:												
Top	24.80	.75	23.60	.91	23.50	.85	21.10	.88	21.35	.82	19.90	.74
Middle	23.80	.72	23.95	.98	23.95	.60	19.95	1.26	21.15	.82	20.35	.58
Bottom	24.65	.94	24.15	.78	23.60	1.02	20.70	1.16	21.85	.67	21.55	.90
No. 25:												
Top	18.80	1.25	19.85	1.27	19.55	.76	15.55	.55	17.40	.46	15.55	.68
Middle	19.85	.71	20.25	.64	19.10	.70	16.60	.70	16.90	1.10	17.05	1.01
Bottom	20.10	.70	20.20	.54	18.40	1.17	16.60	.84	17.75	.64	17.85	1.89

Table 5.—*Analysis of variance for warpwise and fillingwise breaking-strength values for the worn material containing various proportions of new and reclaimed wool*

Variation due to—	Degrees of freedom	Warp	Filling
		Mean square	Mean square
Blends	3	3,702.1063**	2,986.9981**
Replications [1]	81	.7005	1.3892
Skirts	2	3.2646	11.7750**
Sections	2	6.3812	41.7250**
Blends × skirts	6	9.4451**	4.2676**
Blends × sections	6	1.6896	4.0259**
Blends × skirts × sections	12	2.0108	2.5370*
Skirts × sections	4	.8677	3.8625*
Error	243	2.1077	1.1458
Total	359		

[1] Replications and interactions of replications and skirts and sections grouped.
* *F* value exceeds the 5-percent level of significance.
** *F* value exceeds the 1-percent level of significance.

From the latter it is seen that the range in warpwise breaking-strength values for the skirts as a whole and for sections of the skirts is insignificant. However, the variation of the fillingwise breaking-strength values among skirts and among the sections of the skirt is highly significant. Evidently variation in individual wearing habits was insufficient to cause noticeable differences among warpwise breaking-strength values for skirts made of the same blend, while these same differences in wearing habits caused a significant difference in the fillingwise strength. The same is true for the warpwise and fillingwise strength values for various sections of the skirts. Because of the greater initial strength of the warp yarns, it is possible that the amount of wear accorded the garments was insufficient to alter the warpwise strength.

The 81 degrees of freedom for the replications given in table 5 include 9 degrees of freedom for replication alone, 18 for replication × skirts, 18 for replication × sections, and 36 for replication × skirts × sections. These have not been separated into their component parts because each represents a controlled portion of the variance due to fabric variation. The resulting mean square for both warpwise and fillingwise strength is nonsignificant. Thus that part of the fabric variation that can be controlled and eliminated in the calculations is small.

The highly significant mean square for fabrics made of blended wool indicates that the average breaking strength for both warpwise and fillingwise directions was significantly different. Use of the *t* test (*14*) shows a consistent and progressively significant decrease in breaking strength of fabric No. 100 through No. 25, warpwise and fillingwise.

Since mean square for interaction of blends × skirts, both warpwise and fillingwise, has been shown to be significantly greater than the mean square for error, it is apparent that, on the average, the relative performance of the blends was not the same for all skirts. The same is true for interaction between blends and sections for the fillingwise breaking strength. However, the fact that the interaction of blends × skirts and blends × sections did not exceed, significantly, the second-order interaction, i. e., interaction among three variables, blends × skirts × sections, indicates that the differential responses in both skirts and sections are not likely to be reproducible.

The statistically significant second-order interaction, blends × skirts × sections for the fillingwise breaking strength shows that the relative performance of these blends in different skirts was not the same in all sections. Since the mean square for blends was greater (1 percent level) than the mean square for both interaction of blends × skirts and blends × sections, it is indicated that blend performance was consistent enough to outweigh the influence of individual skirts and sections. From this we may be sure that these blends would rank in the same order in future service.

In general, an increase in warpwise breaking strength for fabric blends No. 100 and No. 25 was evidenced in comparisons of values for fabrics before wearing with those for the worn material from three sections of the garments. However, in two instances for each blend, the middle skirt section and the bottom skirt section, there was a significant decrease in warpwise breaking strength. Rather consistent fillingwise breaking-strength increases are seen for blends No. 100, 75, and 50. The top skirt section, both warpwise and fillingwise, shows fewer consistent significant differences between the unworn fabric and the material from garments after wear and dry cleaning than do the other two skirt sections. Comparison of warpwise and fillingwise breaking-strength values for stored fabrics with those for material from the worn garments reveals few statistically significant differences.

These fabric-strength increases result, probably, from partial matting or felting of the wool fiber and yarn, in addition to some slight shrinkage (as indicated by yarn count), which accompanies wear and dry cleaning. One thousand hours of wear and seven dry cleanings were insufficient to cause much loss in the breaking strength of the four fabric blends in this study.

Differences in strength between front and back skirt sections after wearing were evidenced in several cases by fairly consistent, positive or negative deviations from the mean breaking-strength values for the top, middle, and bottom skirt sections. It should be noted that the trends mentioned are substantiated only by examination of the data and not by statistical analysis, but the fact that these trends are consistent justifies their consideration. From warpwise and fillingwise breaking-strength values for the garments made of fabrics No. 100 and 25, it can be demonstrated that the top front sections were less strong than the top back, while the bottom back sections were less strong than the front. The bottom front sections of blend No. 50 were consistently lower in breaking strength than the bottom back sections. Values for the middle sections of all garments of all blends showed no constant relationship.

Treating the front and back of the skirts as separate units, fabrics No. 100 and No. 25 gave evidence of an equal number of cases where either front or back strength was superior, while in the majority of cases for blend No. 75 the back exceeded the front in strength. The reverse was true for blend No. 50.

All in all, there is some evidence that, for the blends studied, the top front section of the skirt received more wear than the top back section, while the reverse was true for the bottom sections. Examination of the data reveals no consistent trend as to whether the skirt front or skirt back may be described as giving lower breaking-strength values or as to whether a specific blend reacts consistently toward the imposed wearing conditions as regards skirt sections.

Analysis of variance of the bursting strength of the material from the worn garments (table 6) indicates a highly significant difference between the fabric blends. Apparently little variation was due to individual wear habits or to possible interaction between fabrics and skirts. It should be noted that by not grouping mean square of replication and replication × individuals, variation due to replication alone is significant at the 5-percent level, suggesting either a difference in fabric bursting strength in various parts of the skirt or inadequate sampling. The latter inference is not wholly unexpected because of the sampling procedure arbitrarily chosen.

TABLE 6.—*Analysis of variance of bursting-strength determinations of the worn garments containing various proportions of new and reclaimed wool*

Variation due to—	Degrees of freedom	Mean square
Blends	3	3,527.7806**
Replications [1]	27	7.6782
Skirts	2	6.8812
Blends × skirts	6	9.9368
Error	81	5.5714
Total	119	

[1] Replications and replications × skirts grouped.

** = *F* value exceeds the 1-percent level of significance.

One thousand hours of wear plus seven dry cleanings resulted in a marked loss in bursting strength for fabrics from the three garments made of 100 percent new wool (table 7). The loss for fabrics No. 75 and 50 was nonsignificant, while only one garment made of fabric No. 25 showed a real loss in fabric bursting strength. However, there still exists the marked difference between the four blends after wear. Actual bursting-strength losses due to wear and dry cleaning range from 5.15 pounds for skirt, No. 1 and No. 2 of blend No. 100 to 1.15 pounds for skirt No. 2 of blend No. 25. It is interesting to note that the average loss in pounds for all blends decreases progressively, 5.03, 2.97, 2.65, 2.60 for fabrics No. 100 to No. 25, respectively.

TABLE 7.—*Yarn-count, thickness, bursting-strength, and bursting-elongation values for the worn materials containing various proportions of new and reclaimed wool, 1942*

Fabric and skirt identification	Yarn count		Thickness		Bursting strength		Bursting elongation	
	Warp	Filling	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
No. 100:	*Number*	*Number*	*1/1,000 in.*	*1/1,000 in.*	*Pounds*	*Pounds*	*Pound*	*Pound*
Skirt No. 1	44.9	40.7	37.55	1.01	75.95	2.44	0.25	0
Skirt No. 2	44.0	40.6	37.95	.55	75.95	2.75	.25	0
Skirt No. 3	45.1	40.6	38.05	1.00	76.30	3.50	.25	0
No. 75:								
Skirt No. 1	44.0	39.6	38.50	1.62	66.25	2.68	.25	0
Skirt No. 2	43.8	39.4	38.10	.70	66.25	2.34	.25	0
Skirt No. 3	44.5	39.7	39.20	.92	67.30	2.45	.25	0
No. 50:								
Skirt No. 1	43.7	39.4	37.15	1.55	59.05	2.06	.25	0
Skirt No. 2	43.5	39.1	37.25	1.11	59.05	2.34	.25	0
Skirt No. 3	43.4	39.3	36.40	.88	58.15	1.73	.25	0
No. 25:								
Skirt No. 1	42.8	39.4	35.75	.98	51.25	2.71	.25	0
Skirt No. 2	42.8	39.0	35.55	.86	52.20	1.90	.25	0
Skirt No. 3	43.0	39.2	35.00	.97	48.80	2.24	.25	0

Bursting elongation after wear was the same for all fabric blends. The small decrease evidenced between bursting elongation of dyed fabrics before and after wearing was not statistically significant.

As may be seen from tables 8 and 9, the highly significant mean square for blends, as shown by an analysis of variance of both warpwise and fillingwise strip-elongation determinations, indicates real differences between the fabric elongation for the four blends. Fabric elongation differed in various skirt sections, both warpwise and fillingwise, although variation in elongation in skirts of the same blend was significant only in the filling direction. Since for both warp and filling directions, the interaction of blends × skirts and blends × sections is significant (1-percent level), fabric elongation variation for each blend is not the same for all three skirts and all three sections. In general, these first-order interactions exceed the second-order interaction, blends × skirts × sections, only at the 5-percent level, thereby allowing controversial interpretation as to the significance of reproducibility of results in this case.

The skirt measurements taken before and after each dry cleaning reveal that the skirt dimensions, with very few exceptions, either increased or remained the same throughout the wearing period. It appears that wear tended to offset any permanent shrinkage which might be caused by dry cleaning.

TABLE 8.—*Means and standard deviations of warpwise and fillingwise fabric-elongation determinations in inches for 3 sections in each of 3 skirts made of blended fiber, 1942*

Fabric No. and skirt section	Warp						Filling					
	Skirt No. 1		Skirt No. 2		Skirt No. 3		Skirt No. 1		Skirt No. 2		Skirt No. 3	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
No. 100:												
Top	0.81	0.06	0.80	0.05	0.84	0.07	0.81	0.10	0.80	0.07	0.87	0.07
Middle	.86	.05	.84	.07	.88	.04	.92	.06	.87	.05	.92	.06
Bottom	.94	.05	.89	.06	1.00	.08	1.01	.06	.93	.08	.98	.10
No. 75:												
Top	.72	.06	.72	.06	.73	.07	.70	.10	.80	.07	.81	.06
Middle	.78	.04	.75	.05	.79	.06	.81	.03	.79	.03	.87	.05
Bottom	.81	.06	.85	.08	.83	.07	.81	.06	.85	.05	.93	.05
No. 50:												
Top	.67	.07	.62	.06	.65	.07	.72	.03	.70	.05	.67	.05
Middle	.64	.05	.65	.05	.62	.04	.67	.05	.71	.03	.72	.06
Bottom	.71	.03	.69	.03	.69	.06	.73	.05	.75	.07	.70	.07
No. 25:												
Top	.51	.06	.53	.07	.51	.03	.54	.05	.59	.06	.55	.05
Middle	.52	.04	.55	.05	.50	.05	.59	.06	.63	.07	.61	.03
Bottom	.56	.05	.55	.05	.53	.05	.64	.07	.59	.06	.62	.06

TABLE 9.—*Analysis of variance of warpwise and fillingwise fabric elongation for the worn materials containing various proportions of new and reclaimed wool*

Variation due to—	Degrees of freedom	Warp	Filling
		Mean square	Mean square
Blends	3	1.9883**	1.5956**
Replications [1]	81	.0037	.0043
Skirts	2	.0037	.0220**
Sections	2	.1952**	.2054**
Blends × skirts	6	.0130**	.0273**
Blends × sections	6	.0177**	.0200**
Blends × skirts × sections	12	.0038	.0079*
Skirts × sections	4	.0009	.0070
Error	243	.0030	.0036
Total	359		

[1] Replications and interactions of replications and skirts and sections grouped.
*=*F* value exceeds the 5-percent level of significance.
**=*F* value exceeds the 1-percent level of significance.

Variations in yarn count among the unworn dyed fabrics and fabrics from the 12 skirts differed no more than 1 to 1½ yarns per inch for either warp or filling directions, indicating relatively stable yarn count regardless of the imposed wearing conditions, as would be expected.

At the end of the total wear period materials from all garments had increased in thickness, although not significantly more than the increase observed during the storage period. Fabric No. 25 continued to be the thinnest fabric blend.

SUMMARY

Four flannel fabrics containing various proportions of new wool of known history and high-quality sweater clips were made into 12 four-gore skirts and issued to women students at South Dakota State

College for a wearing period including 1,000 hours of wear and 7 dry cleanings.

Physical and chemical tests were made on the blended fabrics after they were received from the dyers, after the total wearing period, and after a storage period equivalent in time to the skirt wearing period. It should be emphasized that the data and interpretation reported herein pertain only to fabrics made of the particular quality of new and reclaimed wool fiber used in this study.

Percentage loss, based on the strength of the 100 percent new-wool fabric, in warpwise breaking strength which resulted from the blending of new and reclaimed wool used in this study ranges from approximately 12 percent for fabric No. 75 to 46 percent for No. 25. Fillingwise breaking-strength and bursting-strength values also reveal significant strength decreases throughout the series from fabric No. 100 to No. 25.

Increases in fabric thickness and in certain breaking strength determinations and decreases in certain bursting-strength determinations were observed when comparing values for stored material with those for the material before storing. Other physical measurements for the stored materials varied within the limits of sampling error.

Both nitrogen and sulfur content, calculated on a moisture-free basis, tend to decrease for the dyed fabrics containing large percentages of reclaimed wool; however, this decrease does not hold consistently for the stored and worn blended fabrics. A decrease in nitrogen and sulfur was found following the storage and the wearing periods as compared to the corresponding newly dyed fabrics. The total ash content for all blends increased after the wearing periods. Evidences of chemical deterioration appear to be more pronounced after the wearing period than after storage.

Analysis of variance of breaking-strength values for the skirt fabrics gave evidence that variations in individual wear habits were not sufficient to cause noticeable differences among warpwise breaking-strength values for fabrics made of the same blend, while a differential influence of wear on the filling yarns was noted. The relative performance of the blends was not the same for all skirts or for all skirt sections. However, the differential responses for both skirts and sections are not likely to be reproducible. It is demonstrated that blend performance was consistent enough to outweight the influence of individual skirts or sections. One thousand hours of wear and seven dry cleanings were insufficient to cause much loss in breaking strength of the four fabric blends used in this study, but actual bursting-strength losses due to wear and dry cleaning ranged from 5.15 to 1.15 pounds. Fabric elongation, as measured by both warpwise and fillingwise strip-elongation determinations on the worn fabrics, decreased significantly from fabric No. 100 to No. 25.

The statement that wool fibers reclaimed from unused knit material are not damaged sufficiently "to seriously diminish their original, natural intrinsic protective and service qualities" (*1*) is not supported by these findings.

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DETERMINING NET RAINFALL UNDER A CONIFER FOREST[1]

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INTRODUCTION

It is generally accepted that considerable quantities of rainfall and snowfall are intercepted by tree crowns in the forest. Where water supply or flood control is a crucial factor in regional economy, however, those responsible for management of watersheds need to know more exactly how much of the total precipitation reaches the ground beneath any forest canopy, and to what extent this quantity is augmented by removal of trees in timber-cutting operations. In research on methods of watershed management, measurement of unintercepted or "net" precipitation is indispensable. Since controlling conditions may vary considerably within a single forest-type zone in any given region, this measurement presents a rather involved problem in sampling.

Interception of precipitation by trees has been studied by a number of investigators during the past 60 years and more, but practically all of this research has been based on measurements referred to individual trees. Estimates of effective or net precipitation per acre have been obtained only by conversion. Results of the earlier work on interception, especially that done in Europe, have been summarized by Zon (*11*)[4] and Horton (*2*).

Among more recent results are those detailed by Horton (*2*), who studied interception by open-grown and hedge trees of various species in New York State. There he found that the initial storage capacities of individual trees ranged from 0.02 to 0.07 inch of precipitation. Pines and hemlock stood midway in the scale, which included species of willow, basswood, maple, oak, horsechestnut, beech, apple, ash, elm, and hickory. Net rainfall under the trees ranged from none, for observed storms below 0.07 inch, to about 75 percent of the total in large storms of long duration.

Other recent studies include those by Beall (*1*) and Lunt (*4*), and one by Kittredge et al. (*3*) in a young plantation of Canary pine in California. Kittredge found that interception by tree crowns ranged from 17 to 28 percent of the season's precipitation, with an initial storage of 0.02 to 0.04 inch per storm.

In 1938 and 1939 Wicht (*7*) in South Africa attempted to make direct measurements of net precipitation under a forest canopy in a

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[3] Maintained by the Forest Service, U. S. Department of Agriculture, at Fort Collins, Colo., in cooperation with Colorado State College of Agriculture and Mechanic Arts.

[4] Italic numbers in parentheses refer to Literature Cited, p. 512.

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stand of gray poplar (*Populus canescens*). Wicht employed four rain gages and, by moving the cans from place to place, sampled rainfall at a total of 20 sites within his sample plot. Sampling errors were computed from records for rain gages which were located not at random but mechanically along the sides of a 50-foot square. "Gross" rainfall was measured by means of a single gage in the open, 350 feet from the sample plot.

In its watershed management studies in the lodgepole pine type in Colorado, the Rocky Mountain Forest and Range Experiment Station had need for a similarly direct means of measuring the influence of a forest canopy of mature timber on net precipitation. Accordingly, the writer (*9*) in 1938–40 made a detailed study in this type and class of timber of the effect of timber cutting on net rainfall and flow of rainfall along tree stems, excluding the interception of snow, which was the subject of a separate investigation (*8*). The results showed that stem flow was negligible and that cutting in general augmented materially the net rainfall; but the data on influence of cutting intensity were somewhat inconclusive. Minor discrepancies in the effects of treatment as measured in that study were thought to be due to errors of sampling of interception in a forest. For this reason, and because earlier studies of rainfall interception have shown deficiencies in experimental method, the experiment was redesigned and repeated.

The objectives of the second study, conducted in the summer of 1941 and confined to rainfall only, were to provide a check on earlier results and, by means of an improved experimental design, to measure more accurately the influence of cutting intensity upon net rainfall; also, to demonstrate that an efficient design makes it possible to obtain accurate results with a minimum of work and expenditure for equipment. In view of these objectives, the results and conclusions recorded here quite naturally and unavoidably stress method and design equally with the biological and physical aspects of the study.

EXPERIMENTAL SITE AND PLOTS

Together with related investigations in watershed management, this study was conducted on the Fraser Experimental Forest, at the headwaters of the Colorado River near Fraser, Colo. The type is mature lodgepole pine (*Pinus contorta latifolia*), the average elevation 9,300 feet. Weather conditions, topography, and forest cover are typical of the general zone. The plots include considerable variation in slope, exposure, and timber-stand characteristics. The greater part of the annual precipitation on the area occurs as snow. Rainfall during the snow-free period (June through September) ranges from about 3 to more than 10 inches. Individual rainstorms are ordinarily gentle and light, averaging about 0.25 inch and seldom exceeding 1 inch in depth.

The experiment was conducted on 20 plots, each 5 acres in area, grouped in 4 blocks of 5 plots each. In each block, 4 intensities of cutting treatment had been applied in 1940, and the fifth plot was uncut (fig. 1). The 4 kinds of treatment, assigned to as many plots in each group at random, had left commercial reserve stands of 0, 2,000, 4,000, and 6,000 board feet per acre, respectively. By "commercial" reserve stand is meant residual merchantable trees more than 9.6 inches in breast-height diameter. Cutting was restricted to trees above that size; thus even the "clear-cut" plots contained residual stands of small and unmerchantable trees. As a minor treatment, on half of each treated plot the undesirable trees were removed by cutting. The other plots were given no stand improvement.

SOURCES OF VARIATION AND METHODS OF SAMPLING

The considerable variation characteristic of net rainfall under a forest canopy may be divided into two general classes, each having several sources. The first class includes variations common to all observations of net rainfall on a given area or a given plot; the second, variations among individual observations within the area or plot.

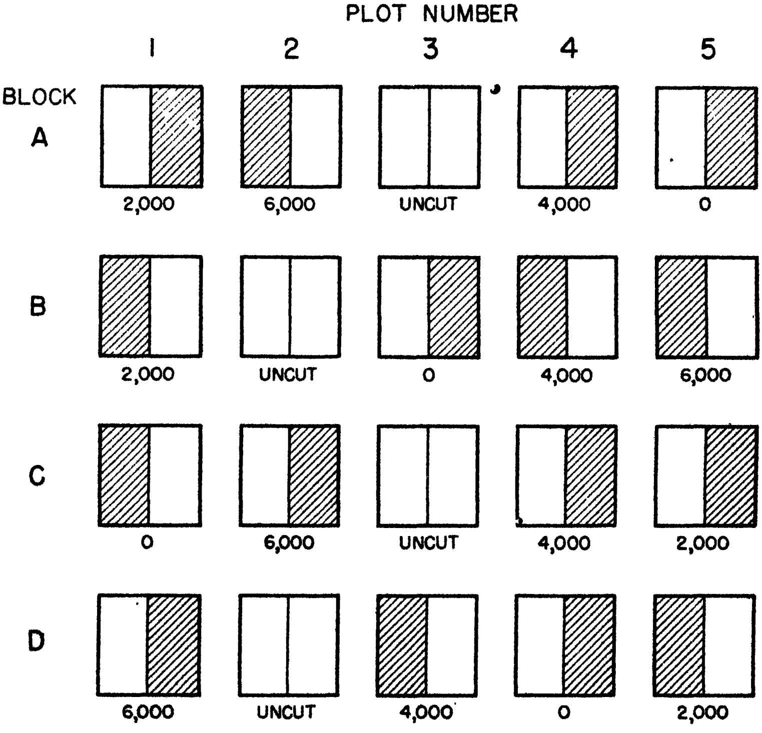

FIGURE 1.—Distribution of treatments, in four randomized blocks. Figures under plot diagrams signify commercial reserve stand per acre in board feet. Hatching signifies timber-stand improvement.

In the present experiment the average net rainfall on any half-plot may deviate from the "true" average—for all possible like-treated half-plots in the universe of mature lodgepole pine—because of local effects of altitude, exposure, and related factors on gross rainfall. It may differ also from averages for other half-plots because of variations in character of the forest from place to place; the various plots and half-plots may not have been alike before treatment, and each treatment may not have left the same residual stand on all the half-plots to which it was applied. Variances (squared standard deviations) due to these sources of variation represent the true interaction of treatment with block—the differential in response of net rainfall to treatment among the several blocks. This interaction affords a strictly unbiased estimate of experimental error only if each block has been located at random in the universe of mature lodgepole pine and if variations among plots in the intensity of a given treatment represent a random sample of all possible variations in that treatment. In practice such conditions can seldom be quite fulfilled; ordinarily, in any experiment such as this, areal limitations preclude random sampling of the universe under consideration. Then the estimated experimental errors are correspondingly limited in general utility, and judgment and conservatism must be used in extending results to other areas.

Sources of variations in the second class are commonly called sampling errors. Since it is impracticable to measure every possible variation in net rainfall on each half-plot, an attempt must be made by sampling to obtain a reasonably accurate estimate of the true average net rainfall. The extent to which the average of the sample will deviate from the true average depends upon the number and distribution of samples and upon the magnitude of variations in net rainfall within the half-plot. These variations (aside from personal and instrumental errors) may arise from three general sources: (1) Variations in forest cover density from place to place within the plot; (2) variations in net rainfall at a single sampling spot, for storms of the same size; and (3) variations in gross rainfall from storm to storm and from place to place within the plot.

The magnitude of each sampling observation will deviate more or less from the true average of net rainfall according to the sizes and directions of the variations arising from these sources. If all the components of variation are random and independent in character, the mean value of each or their joint value will approach zero as the number of plots within the universe and the number of samples taken within a plot increase. The variations themselves may be random and compensating; their squares, on the other hand, are all positive, and the variances due to all components of variation are added together to form the variance for experimental error.

Assuming that all the variations in both classes are random and independent in the probability sense, we may express the magnitude of any single observation by the equation.

$$x_{ij} = P + a + b_1 + b_2 + b_3, \qquad \text{(I)}$$

in which x_{ij} is any single sample of net rainfall at sampling place j within the half-plot i; P is the true average net rainfall for any single treatment; a is a component of random variation arising from all sources common to a single plot or half-plot; and b_1, b_2, and b_3 are the variations of the second class arising from the three general sources, in the order listed above. The true value P cannot be determined; it is possible, however, to estimate the variance of sample observations around the true value by employing the familiar formula (with extensions to include two or more categories of variation)

$$s^2 = \frac{S(x-\bar{x})^2}{n-1}, \qquad \text{(II)}$$

in which s^2 is the estimated variance, S is a sum, x is any sample observation, $\bar{x}$ is the average of all observations, and n is the number of observations. Then, in terms of variances, equation (I) may be expressed as

$$s_T^2 = s_a^2 + s_{b_1}^2 + s_{b_2}^2 + s_{b_3}^2, \tag{III}$$

in which s_T^2 is the variance of a single sampling observation for any one treatment and s_a^2 etc. are variances due to the four components of variation mentioned above. In subsequent discussion these variances will be identified only by their subscripts (capitalized), as "A" for "s_a^2."

If the largest practical number of uniform blocks has been established and the treatments carefully applied, the A component is unchangeable in the magnitude of its influence upon the average net rainfall under each treatment. Since, however, the error variance of any average decreases directly with the number of observations, the influence of the B components may be minimized by taking a maximum number of samples of net and gross rainfall within each half-plot; and B_3 may be largely removed by adjusting net rainfall to values expected for the average gross rainfall of all samples. In addition it is possible, by proper design of the sampling procedure, to evaluate the individual influence of each B component.

For the present study, the objectives of sampling were to obtain treatment averages accurate within approximately 5 percent, and to obtain an unbiased estimate of each component of error.

Very rough estimates of sampling errors derived from earlier studies indicated that rainfall should be sampled at not less than 10 places on each 2½-acre half-plot. According to past procedure 400 pairs of rain gages would be required, one member of each pair to be used for measuring net rainfall and the other for measuring gross rainfall. One season's records would then include roughly 10,000 to 12,000 pairs of observations. Since this number seemed excessive, and since earlier studies had indicated that the errors due to variations in storm size could be largely removed by regression, it was decided to simplify sampling by partial confounding of the B_3 component with the two other components of sampling error within each half-plot. Accordingly 12 rain-gage locations were selected at random for the measurement of net rainfall on each half-plot. Then one pair of 8-inch standard rain gages was assigned to each half-plot. The "net" gage was placed at one of the 12 selected sites, then the gage to be used for measuring gross rainfall was placed in the center of the nearest available opening of a size large enough to free the records from interception effects—at least 30 feet in diameter. After each storm the "net" rain gage was moved to a new site, and the "gross" gage placed in the nearest suitable opening. Thus a series of 12 storms gave 1 complete circuit of the locations on each half-plot. For the succeeding 12 storms, the same locations were used in newly randomized sequence. Thus after 24 storms 2 pairs of readings were available for each of 12 locations on each of 40 half-plots—a total of 960 observations.

Depth of rainfall was recorded in inches. Since the locations for measurement of net rainfall were randomized over each half-plot, the resulting data expressed net and gross rainfall directly in inches per unit of plot area.

METHODS OF ANALYSIS

The study was so designed that analysis of variance and covariance (*5*, *6*) could be applied. The null hypothesis to be tested was that differences in net rainfall associated with the major and minor treatments were no greater than variations, due to uncontrolled causes, which might occur by chance. In simplified terms, the analysis partitioned the total variance (squared standard deviation) of the study into two general classes, the variances between and within groups of data (see table 2). The former, measuring controlled variation, are the variances between major treatments (1), between blocks (2), and between minor treatments (3), and the interaction of major and minor treatments (1×3). The "within" variances, measuring uncontrolled variation, are composed of the several mean squares which form the experimental errors for testing treatment effects. The interaction of major treatment with block forms the experimental error appropriate for testing major treatments. The minor-treatment error includes three variances—the interaction of minor treatment with block (2×3), the triple interaction (1×2×3), and the variance between the halves (both untreated) of each uncut plot. Each of the two experimental errors is the sum of variances arising from all sources of sampling error.

In order to minimize sampling errors due to variation in gross rainfall (B_3), all the variances mentioned above were adjusted by regressions of net rainfall on gross. As a result, each of the two other components of sampling error within plots (B_1 and B_2) has confounded in it the errors of estimate of the regression of net rainfall on gross. In subsequent discussions, the variances expressed by B_1 and B_2 will be understood to include this residual portion of B_3.

As usual, the procedure in analysis of covariance involved simply the computation of average net rainfall per plot and per treatment, adjusted to the average value for gross rainfall, and, for tests of significance, the calculation of mean squares for all components of variation, adjusted by the "error" regressions of net rainfall on gross. Individual storm observations as well as half-plot averages were used in this analysis, in order to provide estimates of sampling errors.

On the assumption that each of the several components of variation contributed a significant amount to experimental error, the valuation of these components for the major-treatment error in the present study may be laid out as follows:

Mean square (errors of estimate):	*Components*
Within rain-gage locations	B_2 (IV)
Between locations within half-plots	$2B_1+B_2$ (V)
Interaction of block and major treatment	$48A+2B_1+B_2$ (VI)

Similar formulas are applicable to the minor treatment. The formula for interaction is simply III modified to fit the mean squares computed in the variance analysis. The "between locations" mean square does not contain the variance A, which is common to all observations within a half-plot, and the "within locations" mean square contains neither A nor B_1, which is common to all observations at a single location.

Each component is a squared standard deviation, and the numerical coefficient of each is the number of observations (k) making up each subclass sum from which the mean squares were computed (*5*;

6, sections 10.13 and 17.8). Thus the "within sites" term is a variance of individual observations around the mean of two readings, *k* being 1; the major-treatment interaction, on the other hand, is a mean square which is 48 times the corresponding variance of whole-plot averages (each average based on observations of 2 storms in each of 12 locations on 2 half-plots—48 observations altogether).

Since the experiment was designed to provide numerical values for each of the above-mentioned mean squares, an estimate of each component of error could be obtained by successive solutions of expressions V and VI (*10*).

RESULTS

When adjusted to the average gross rainfall [5] for all plots, the average net rainfall per storm indicated a strong and consistent effect of the major treatment (table 1). Considering the half-plots which were not subjected to stand improvement cutting, only 68.4 percent of the gross precipitation reached the ground on the uncut plots, but 90.4 percent did so on the clear-cut plots. Values for the other major treatments approximated a straight line between the two extremes. Figures from earlier experiments (*9*) agreed well with these, except that the linear relation of net rainfall to treatment was obscured by experimental error.

In striking contrast with the pronounced effect of the major treatments, influence of timber-stand improvement was apparently lacking. The advantage shown by the "improved" half-plots over the unimproved in net rainfall amounted to only 2.0 percent of the gross rainfall. The nonsignificance of this quantity is demonstrated by two facts: In the 6,000-board-feet treatment, net rainfall was actually greater on the unimproved half-plots than on the improved; and the uncut half-plots showed as much variation among themselves as occurred between the improved and unimproved cut-over half-plots.

TABLE 1.—*Average net rainfall per storm, adjusted to average gross rainfall*[1]

Stand per acre after major treatment (board feet)	Improved half-plots		Unimproved half-plots		Average	
	Inches	*Percent*	*Inches*	*Percent*	*Inches*	*Percent*
11,890 [2]	0.175	70.0	0.171	68.4	0.173	69.2
6,000	.199	79.6	.205	82.0	.202	80.8
4,000	.216	86.4	.209	83.6	.213	85.2
2,000	.220	88.0	.211	84.4	.216	86.4
0	.238	95.2	.226	90.4	.232	92.8
Average	.210	84.0	.204	81.6	.207	82.8
Average for treated half-plots	.218	87.2	.213	85.2		

[1] Net rainfall has been adjusted to an average gross rainfall of 0.250 inch. This average is the base of the percentages.

[2] No major treatment; neither half-plot was improved.

The indications in table 1 are supported by the analysis of variance and covariance (table 2). The differences associated with major treatments were found to be significantly greater than the chance variations expressed by experimental error ($F = .0756 \div 0.00527 = 14.3$; $p. = < 0.01$). Further, each individual treatment resulted in net rainfall significantly greater than that occurring under virgin conditions;

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[5] The average gross rainfall was 0.25 inch per storm; a total of 5.99 inches of rain was recorded in 24 storms between May 22 and September 3, 1941.

TABLE 2.—*Analysis of variance and covariance, net rainfall per storm*

Source of variation	Net rainfall		r^2	Errors of estimate[1]	
	Degrees of freedom	Mean square		Degrees of freedom	Mean square
Total	959	0.0351	0.8825	958	------
Between means of:					
(1) Major treatments	4	.1291**	------	4	0.0756**
(2) Blocks	3	.0247	------	3	.0234*
(3) Minor treatments	1	.0069	------	1	.0068
Interactions:					
(1 x 2)[2]	12	.0087	.4450	11	.00527
(1 x 3)	3	.0055	------	3	.0051
(2 x 3), (1 x 2 x 3), and within uncut plots	[3] 16	.0056	.0460	15	.0060
Within half-plots (sampling error):					
(B^1) Between locations	440	.0359	.8776	439	.00441
(B^2) Between storms within locations	480	.0356	------	475	.00258

Regression formulas:
In interaction 1×2: $E=0.9549\,x-0.0313$; $r=+0.667$.*
In sampling error B_1: $E=0.9667\,x-0.0342$; $r=+0.937$.**
In sampling error B_2: Uncut: $E=0.8046\,x-0.0290$; $r=+0.946$.**
6,000 board feet: $E=0.8677\,x-0.0149$; $r=+0.948$.**
4,000 board feet: $E=0.9055\,x-0.0131$; $r=+0.956$.**
2,000 board feet: $E=0.8933\,x-0.0074$; $r=+0.966$.**
0 board feet: $E=0.9897\,x-0.0153$; $r=+0.993$.**
The B_2 regressions differ significantly from each other ($F=7.236$).

[1] Around "error" regressions of net rainfall on gross rainfall.
[2] Experimental error for major treatment.
[3] Experimental error for minor treatment.

as can be shown by analysis of the single degrees of freedom associated with the treatments (for example, for the 6,000-board-foot treatment $F=13.8$). It is improbable that the magnitude of apparent effects of major treatments was appreciably influenced by the minor treatment, since the interaction of major and minor treatments was no greater than its experimental error.

The mean square expressing effect of minor treatment on net rainfall was only slightly larger than the error mean square.

The results of tests of treatment effects are presented in table 2 in the column "errors of estimate," which shows all effects adjusted to the average size of storm for all plots. The procedure of adjustment materially augmented experimental precision in comparing the major treatments; this is shown by the reduction in size of the error mean square (0.00527 for adjusted as compared with 0.0087 for unadjusted averages). It is sounder in principle than the more common recourse of analyzing net rainfall or interception as a percent of gross rainfall.

Aside from the tests of treatment effects, table 2 presents regression statistics which cast additional light on the influence of major treatments upon net rainfall. Of the numerous "error" regressions within treatments, only those computed for sampling error B_2 varied significantly among the five major treatments. Accordingly an individual regression formula was computed for each treatment; and the B_2 mean square in table 2 is the average of the errors of estimate for the five regressions. By equating each of these formulas to zero, the initial crown storage per acre was computed (with some error) for each major treatment. According to the computations about 0.036 inch of rain had to fall on the uncut stands before any rainfall penetrated the forest canopy. The corresponding figures for the four major treatments, in ascending order of treatment intensity, are 0.017, 0.014, 0.008, and 0.015 inch. By use of these formulas net rainfall on these particular plots can be computed with considerable accuracy for any given storm value. In a storm of 0.60 inch, for example, the net rainfall on the uncut plots would be about 0.45 inch; on the "clear-cut" plots, about 0.58 inch. The error of these predictions may be estimated by use of the formula

$$V_E=\frac{V_{y.x}}{n}+(X-\bar{x})^2\left(\frac{V_{y.x}}{Sx^2}\right),$$

in which V_E is the variance of the predicted value for net rainfall; $V_{y.x}$ is the mean square for errors of estimate (B_2); X is the gross rainfall from which net rainfall is estimated (0.60 inch); $\bar{x}$ is the mean gross rainfall on which the regression was based; and Sx^2 is the sum of squares for x in the regression data. For the uncut plots in a storm of 0.60 inch,

$$V_E=\frac{0.00258}{192}+(0.60-0.250)^2\left(\frac{0.00258}{4.4656}\right)=$$

$$0.000013+(0.1225)(0.000578)=0.000084.$$

The corresponding standard error is ±0.009 inch, about 2.0 percent of the estimated net rainfall of 0.45 inch. Similarly, the error of the computed "initial storage" on the commercially clear-cut areas was found to be ±0.0057 inch; thus the deviation of the computed value (0.015 inch) from the strong trend shown by the other treatments is probably due to chance.

Examination of the mean squares for the several components of error reveals that neither experimental error greatly exceeds the desired limit, 5 percent of the average net rainfall. For major treatments, the standard error of treatment averages is ±0.0052 inch $\left(\sqrt{\frac{0.00527}{192}}\right)$, or about 3.0 percent of the lowest average value per storm for a single treatment (that for uncut plots, 0.173 inch). For minor treatments, the corresponding standard error is 0.0035 inch, or 1.7 percent of the average value per storm (0.204 inch) for the 20 unimproved half-plots.

Since the error of the major-treatment averages exceeded 5 percent (3.0 percent times t at the 0.05 level for 11 degrees of freedom = about 6.6 percent), it may be illuminating to analyze the mean square for the experimental error of major treatments; that is, to determine the magnitudes of its several components and estimate in what way experimental error can most efficiently be brought down to the desired limits. In the present study this analysis was somewhat academic, since the major treatments were shown to have very significant effects and the minor treatment to exert no appreciable influence; however, exposition of the method and evaluation of error components may assist in preparation of efficient experimental designs for future studies.

As has been pointed out (expression VI), the interaction of block and major treatment has three general components of error; its mean square may be expressed as $48A+2B_1+B_2$. Parenthetically, then, the variance of treatment averages may be expressed by the equation

$$V=\frac{\text{mean square}}{192}=\frac{MS}{48\times4}=\frac{A+B_1/_{24}+B_2/_{48}}{4},$$

since the mean square for single plots is 48 times the variance of plot means and each treatment average is based on 4 plots. From available data numerical estimates of each of these three components can now be computed. In table 2, the adjusted mean square[6] for variation "between locations within half-plots" (0.00441) contains variances due to the random error of measurements at a single location ($B_2=0.00258$) and due purely to variation in net rainfall from place to place within a half-plot ($2B_1$, since the average for each location is based on two storm observations) Thus

$$\begin{aligned} 0.00441&=2B_1+0.00258, \text{ and} \\ B_1&=0.00092 \end{aligned}$$

Then the mean square for experimental error may be analyzed as follows:

$$\begin{aligned} 0.00527&=48A+0.00183+0.00258; \\ 48A&=0.00086, \text{ and} \\ A&=0.000018. \end{aligned}$$

Now it can readily be seen, by comparing the magnitudes of B_2, $2B_1$, and $48A$, that the interaction mean square is composed chiefly of sampling errors, which in turn consist chiefly of variations between measurements at a single site. Since the given arrangement of observations failed to yield averages accurate within 5 percent, it is desirable to make one or more of these components of error smaller if possible. The A component is so small that further reduction by the use of additional plots would seem unnecessary; and the B_3 component (due to storm size) has been reduced as far as possible by the regressions of net rainfall on gross. Either of the two other B components can be reduced, however, by increasing the number of locations sampled or the number of storm observations at each location.

Assuming that the several components have been estimated with reasonable accuracy, it is not difficult to work out the arrangement of storm observations which is likely to reduce most efficiently the error of treatment averages. The first step is to ascertain the most efficient arrangement of samples; that is, to estimate what number of observations at each location will provide maximum accuracy with least work. For this purpose, use may be made of the formula $k^2=\frac{c(B_2)}{(B_1)}$, in which k is the optimum number of samples to be taken at a single location and c is the number of samples which can be taken at each place in the time required to move to a new location and set up the rain gage for a new reading. In the present study c may be taken as unity, because it is believed necessary to move gages after each storm. Then,

$$k^2=\frac{0.00258}{0.00092}=2.8043, \text{ and } k=1.67,$$

or, in round numbers, two observations at each location. Thus, in order to reduce the error of treatment averages to the desired limits with a minimum of work, it is desirable to sample additional locations rather than take more samples at each of the 24 places within each whole plot.

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[6] Unadjusted mean squares could not be used in this analysis. Since storm size was assigned at random only to individual samples within half-plots, the unadjusted mean squares for sampling errors are much larger than the corresponding mean squares for the two experimental errors, to which storm size was not randomly apportioned

Now the number of locations required to provide the desired standard error (about ±0.0043 inch) may be estimated by solving the interaction formula equated to k_1, the desired number of locations·

$$k_1=\frac{B_1+B_2/_2}{4V_{\bar{x}}^2-A}.$$

In this case

$$k_1=\frac{0.00092+0.00129}{0.000074-0.000018}=39.46,$$

or about 40 locations.

By another solution of the same formula it is possible to demonstrate the greatly increased sampling efficiency obtained by moving gages between storms instead of using gages left in the same place for an equal number of storms. If 40 storms were measured with each pair of gages, for example, about 340 pairs of gages at fixed locations (at least 17 pairs per whole plot) would probably be required for the experiment in order to provide treatment averages accurate within 5 percent; or, if 24 storms were measured with each pair, well over 200 pairs of gages at fixed locations would be necessary to attain the present accuracy of 6.6 percent. In the first of these two examples, the calculation was as follows·

$$k_1=\frac{0.00092+0.00258/40}{0.000074-0.000018}=\frac{0.000984}{0.000056}=17.5,$$

or, in round numbers, 17 pairs of gages per whole plot.

The relative efficiency of the recommended sampling method is obvious when the labor and equipment required by the alternate procedure are considered. With rain gages at fixed locations, the necessary investment in gages alone would be prohibitive for most studies— about $2,000 (at $3 per instrument) as compared with $240 for the 80 gages employed in the present experiment. The labor of reading 680 rain gages would greatly exceed that of reading and moving 80 gages; and the effort required for compiling and analyzing 27,000 individual storm observations would hardly be pleasant to contemplate.

SUMMARY AND CONCLUSIONS

Analysis of the results of the experiments described here reveals a significant relation between degree of timber cutting and amount of rainfall reaching the ground. Cutting mature lodgepole pine to a commercial reserve stand of 6,000 board feet or less per acre will cause significant increases in net rainfall. The event of increase of tree reproduction and other vegetation will of course alter this relation. Net rainfall appears to vary in linear relation to the intensity of cutting. The analysis indicates, however, that removal of undesirable trees, that is, the described timber-stand improvement work, resulted in no appreciable increase in net rainfall. Presumably variations in stand density that are not readily measurable obscure the effect of improvement cuttings.

Regarding the experimental design and the statistical method employed in the study described here, it is evident (1) that the design is satisfactory for measuring at least major treatment effects, and is very much more efficient than previously used methods of sampling; and (2) that in any sampling of rainfall interception, maximum efficiency can be expected to result from taking two pairs of storm observations at each of a number of randomized sampling locations. The latter conclusion should, of course, be verified by actual trial in any new experiment.

For new studies it is recommended that sampling of net rainfall be designed to provide randomized measurements at a maximum number of locations with the least practicable number of storm observations—usually two—at each site. In any experiment measurements should be taken in two or more seasons, so as to obtain an estimate of the interaction of treatment with season. Unless the number of storms occurring in a single season makes it possible to sample rainfall at a number of locations sufficient to minimize sampling error, it may be desirable to use a new set of randomized sites for each season's work. With this modification and any others needed to fit local conditions or different sampling problems, the experimental design employed should prove satisfactory in other forest types. The general principles and procedure in the analysis of sampling errors may be applied also in other kinds of sampling experiments.

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